EFFECTS OF CAPTIVITY ON FORAGING BEHAVIOR AND SURVIVAL IN THE WILD OF *MICROTUS PENNSYLVANICUS*

By Amaranta E. Kozuch

Human activity has caused wildlife populations worldwide to decline making it imperative for conservation biologists to develop captive breeding and reintroduction programs. These programs, however, have had limited success. Captivity has been shown to select for behavioral traits that are maladaptive in the wild, such as inability to recognize optimal food resources, thereby minimizing survival. I developed this study to explore the mechanisms involved with behavioral change in a systematic and hypothesis-driven framework. I captured, housed and later tested meadow voles (*Microtus pennsylvanicus*) in a foraging test to measure behavioral differences as a function of environment and time. Animals were housed in either a simple or complex environment for greater than or less than 1.5 months. Analysis of behavioral data from the foraging test suggests the complex environment may maintain appropriate foraging behaviors for sexes and a short time in a simple environment may maintain appropriate responses to unpredictability. All subjects along with a wild cohort were subsequently released into outdoor enclosures and survival was monitored. Analysis of mark-recapture data suggests environment and time do affect survival and recapture of individuals differently; animals housed in complex environments (for less than 1.5 months) maintained similar survival rates as wild individuals. My study suggests that captive individuals may benefit from additional complexity (for short time intervals) within the captive environment to maintain wild behaviors and increase survival upon reintroduction.

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by

Amaranta E. Kozuch

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COMMITTEE APPROVAL

_____ Advisor

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_____ Member

15 May 2012 Date Approved

_____ Member

5/16/2012 Date Approved

PROVOST
AND VICE CHANCELLOR

_____

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Dedications

To my mom and bro, Sasa baby, the voles, and the irreplaceable beauty of Nature.

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Chapter I
The Importance of Conservation Behavior

Species diversity plays a key role in ecosystem maintenance (Seddon, 1999) because greater diversity increases an ecosystem's ability to resist anthropogenic disturbance. Although, species are faced with a number of anthropogenic conflicts, including fragmentation of habitat by roads, poaching for black market products, altering food resources through farming, pollution from human activities, and invasive species introduction both accidentally and intentionally (Anthony and Blumestein, 2000). Anthropogenic impacts have increased the loss of biodiversity and species need our help to reverse the damaging effects of our actions. As species' populations are being impacted dramatically, conservation biologists are working to protect biodiversity through management and study of population trends and interactions within ecosystems (Caro, 1999, Soule, 1985). However, there are limitations to management based on conservation biology theories alone, mainly because it is a crisis discipline (Soule, 1985). Conservation biologists solve situations with wildlife species quickly and with limited knowledge of species' biology. Therefore hypothesis-driven studies testing the effects of different conservation methods can guide managers to develop efficient conservation plans when in crisis. For example, the use of a 'soft' release procedure during reintroductions to minimize stress and increase survival probability (Letty, 2000,

Teixeira et al., 2007), and minimize loss of an already small number of remaining individuals.

Most conservation studies never incorporate behavioral knowledge or attempt to include behavioral studies in species management (Angeloni et al., 2008) because conservation biology is focused on populations, and ethology is focused on individual variation (Caro, 2007). However, conservation management of species has failed on numerous occasions because of the lack of knowledge on how anthropogenic activities impact an animal's ability to mate, forage, avoid predators, and respond to unpredictability and environmental change (Snyder et al., 1996). These behaviors directly affect species' survival and reproduction.

Behavioral studies can provide conservation biologists with information on proximate and ultimate causes of behavioral expression (Buchholz, 2007) and adaptations in changing environmental conditions. Individuals comprise a population, and wide-spread behavioral change in individuals can shift the frequency of behavioral trait expression over the whole population. If a majority of individuals exhibit inappropriate responses, then population growth may be negatively affected (Anthony and Blumstein, 2000), thereby reducing the probability of establishing self-sustaining populations (McPhee and Silverman, 2004).

Behavioral changes that affect conservation management are evident when animals are bred in captivity and released into the wild (Angeloni et al., 2008). Studies

on behavior play an important part in enhancing captive-breeding methods because they help inform managers of the effects of the captive environment (e.g. restricted space, reduced social interaction, inability to meet behavioral needs, and food availability) on species' welfare and survival in the wild (Hosey, 2005, Jensen and Toates, 1993). The theory that environment affects behavior and behavioral traits affect fitness and survival (Darwin, 1868) should be applied when the goal is to maintain naturalistic behaviors in species housed in captivity. Maintenance of naturalistic behaviors that would be observed in the wild can increase integrity of laboratory research and minimize variability due to a novel environment (Baumans, 2011). If natural behavioral repertoires are maintained, they can be analyzed in the zoo environment, and inform species management when animals cannot be observed in the wild (Olsson and Dahlborn, 2002).

My research focuses on preserving and managing foraging behavior in captivity because an appropriate foraging repertoire is indispensable for survival in the wild and population growth (Berger-Tal et al., 2011). I examined changes in foraging behavior of meadow voles (*Microtus pennsylvanicus*) when exposed to environmental complexity, as defined by cage size and exposure to novel objects, to promote need-based activities such as nest building, burrowing, gnawing, and problem-solving. I compared differences in foraging behavior between individuals housed in a simple and complex environment

to their survival probability after being released into the wild to understand whether differences promote or inhibit survival.

Chapter II

Effects of Environmental Complexity and Time Spent in Captivity on Foraging Behavior of Meadow Voles

Introduction

Expression of appropriate behaviors is fundamental to an animal's fitness and results from an interaction of multiple internal and external factors (Jensen and Toates, 1993). The external environment is stochastic, and an individual's behavior must be plastic to appropriately respond to changing conditions. Adjustments in behavior can occur within an individual's lifetime, either immediately to compensate for experiences or through learning during development (McPhee and Carlstead, 2010). When behavior is affected by varying selective pressures in changing environments, optimum variance and mean expression of critical behavioral traits can undergo shifts (Endler, 1986). Along with physiology and morphology, behaviors can be selected for by varying selection pressures in an individual's environment (Darwin, 1868, Wallace et al., 2000). This can leave individuals ill-equipped to appropriately respond to natural stimuli (McPhee, 2004a), such as predators, food availability, and social interactions. There are three types of shifts (Figure 1) affecting behavioral traits in populations as a result of changes in environmental conditions: directional, relaxed, and disruptive selection (Endler, 1986). Most commonly in captive settings, behavioral variation results from relaxed

selection, where traits that typically fall outside the range that confers fitness in the wild are not selected against (Figure 1B; McPhee, 2004a, Watters and Meehan, 2007). Understanding the mechanisms responsible for altering behavior is extremely important for designing effective management strategies for wild species in changing environments, captive species, future conservation efforts for all species, as well as for scientific integrity.

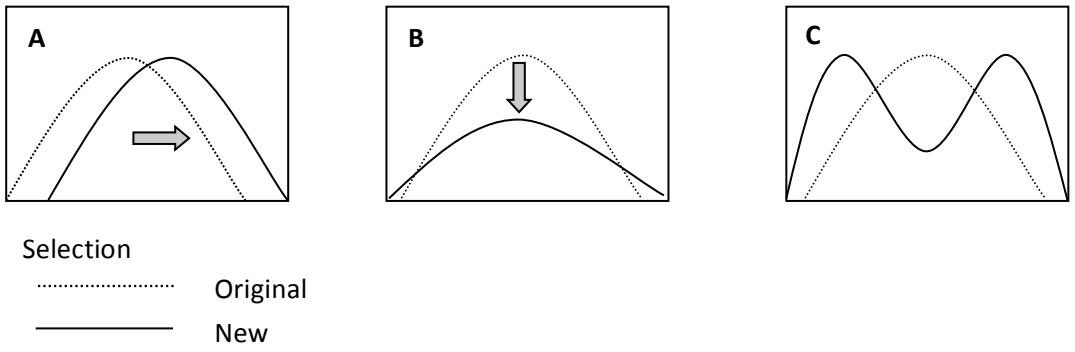

Figure 1: Three types of selection. A) Directional selection, B) Relaxed selection, C) Disruptive selection. X-axis is trait expression, y-axis is frequency.

The captive environment is well known for affecting the behaviors of wild individuals (Biggins et al., 1998, Mathews et al., 2005, Stoinski and Beck, 2004) because of differences in factors such as resource availability, experiences during development, human contact, and lack of space (Kohane and Parsons, 1988). Studies document abnormal behavioral repertoires in zoo animals (McPhee, 2002, Morgan and Tromborg,

2007), and experimental studies suggest that this is a result of stress from an inability to express naturalistic behaviors (Jensen and Toates, 1993, Olsson and Dahlborn, 2002, Hosey, 2005). Individuals in the wild are constantly exposed to an element of unpredictability, which is thought to promote flexibility in their behavioral repertoire (Watters and Meehan, 2007). This stochasticity forces individuals to make choices (Poole, 1998); incorrect decision-making can be detrimental to survival, leading to selection against these individuals. Providing challenging and behaviorally-relevant activities to elicit expression of naturalistic behaviors may improve welfare of animals (Baumans, 2011) and maintain skills adaptive to the wild (Stoinski and Beck, 2004). However, identifying and replicating key elements of the wild environment in a captive setting may be difficult because the wild environment is composed of infinite variables (Poole, 1998) that interact with each other. Can modifying the captive environment provide the necessary biological substance to promote natural behaviors that zoo and conservation biologists aim to maintain?

To begin answering questions such as this, we must understand how the captive environment alters specific behaviors and subsequently how to maintain those behaviors. There are three reasons maintenance of natural behaviors is imperative: reintroduction success (Watters and Meehan, 2007), animal welfare (Veasey et al., 1996), and integrity of research (Baumans 2011, Olsson and Dahlborn, 2002). Reintroduction is an important management tool used to conserve species diversity by

bringing wild animals into a captive environment temporarily or for multiple generations before being released back into the wild. Housing wild individuals for a short period of time before releasing them into the wild is called relocation and may in some cases be a more feasible management tool than reintroducing captive-born individuals (Griffith et al., 1989). The effects of temporary housing in captivity during relocation on behavior have not been fully studied. Rapid changes in behavioral expression have been documented in wild individuals that have been housed in captivity due to stress, and such individuals have been characterized as having poor physical health (Morgan and Tromborg, 2007). However, adding structural complexity to the captive environment has been shown to stimulate and recover expression of natural behaviors immediately (Kitchen and Martin, 1996, Ulyan et al., 2006), but no study has measured the implications that these rapid changes have on a repertoire of behaviors.

Captivity promotes deterioration of the range of behavioral expression and inhibits the development of certain skills (Burrell and Altmann, 2006). The lack of challenges within the captive setting prohibits adaptation to dynamic situations found in the wild, which may limit an individual's repertoire and response time (Beck et al., 2002); in addition, stereotypic behaviors often develop to cope with absent environmental elements (Morgan and Tromborg, 2007). Adding complexity to the captive environment maintains wild behaviors and trait variation (Beisner and Isbell, 2008, Hosey, 2005, Watters and Meehan, 2007). Numerous studies conducted on a

variety of species provide support for designing a captive habitat that incorporates complex and dynamic characteristics (Beck and Castro, 1994, Beck et al., 2002, Burrell and Altmann, 2006, Novak et al., 1994). These incorporated elements have been described as environmental enrichment, which provide a valuable stimulus to deter aberrant behaviors and elicit the appropriate expression of wild behaviors (Carlstead and Shepherdson, 1994, McPhee, 2002). Environmental enrichment, however, has been haphazardly implemented, and not all enrichment promotes behaviors in a biologically relevant manner (Newberry, 1995, Watters and Meehan, 2007). Therefore careful study of environmental elements must be conducted to determine the utility and significance of complexity (Meehan and Mench, 2007).

The difficulty in isolating specific behaviors on which to focus is that a combination of behaviors may be necessary to survive in natural situations. However, focusing on specific traits is necessary to uncover underlying mechanisms for changing behaviors because an environment may affect one behavior more than another. Determining which trait to measure depends on the environmental situation; for instance, large felids spend a small but important portion of their time hunting, and therefore researchers should focus on behaviors associated with finding and capturing prey, such as traveling long distances. If an environment provides a challenge for these individuals to locate food, the environment is still lacking important behavioral “needs” (Jensen and Toates, 1993), such as roaming long distances in search of prey, and thus

may lead to stereotypic behaviors such as pacing. Therefore, all of the behaviors included in the hunting behavioral repertoire are important to maintain.

Animals in the wild are almost constantly involved in activites such as foraging, roaming, and socializing. In captivity, many of these activities are minimized or absent (Morgan and Tromborg, 2007). To design practical habitats within captivity that promote welfare and, later, survival if reintroduced into the wild, we must determine whether different environmental conditions in captivity affect behavioral repertoire and flexibility differently. Changes in foraging behavior have been documented in numerous studies of species held in captivity (Hansen and Berthelsen, 2000, Stoinski and Beck, 2004), and captive herbivores spend significantly less time foraging and more time expressing disadvantageous behaviors (Beissner and Isbell, 2008, Burrell and Altmann, 2006) than wild counterparts.

For this study, I focused on foraging behavior. Ability to forage requires individuals to locate, harvest, and consume food items. Efficient foraging behavior requires complex decision-making that promotes optimality in an individual's activities and can be fatal otherwise. Thus an individual must make particular decisions that are presumed to be optimal in terms of energy intake versus costs (such as predation) (Stephens and Krebs, 1986). Foragers have limited knowledge of environmental quality prior to patch exploration. Thus, an individual must forage to maximize net acquisition rate for energy while minimizing risk to predation and energy shortfall. This complex

behavior to obtain food resources is considered a motivated behavior elicited by internal factors, and an inability to perform feeding behaviors in captivity can greatly affect the animal's welfare (Jensen and Toates, 1993).

To study captivity effects on foraging behavior, I used the meadow vole (*Microtus pennsylvanicus*) as my model species. For this species, reproduction and space use can be greatly affected by one's potential to locate food (Fortier and Tamarin, 1998, Jones, 1990). Voles are short-lived animals that are abundant in my study area. Their abundance allows for easier access for use in research than an endangered or threatened species and provides larger sample sizes for more robust results.

In the field of animal behavior, the hypothesis that the environment affects behavior is widely known and accepted (Beisner and Isbell, 2008, Daan and Tinbergen, 1997, Darwin, 1868). To test this hypothesis within the context of captive-breeding, I examined the foraging behavior of voles housed in two different environments. Based on previous studies (captive felids, Carlstead, 1994, rabbits *Oryctolagus cuniculus*, Hansen and Berthelson, 2000, and golden lion tamarin *Leontopithecus rosalia*, Stoinski et al., 2003), I predicted that individuals held in a complex environment would show differences in foraging repertoires when compared to individuals held in a simple (control) environment. This would indicate that different environments will promote maintenance of different behaviors. A second objective was to examine how foraging behavior was affected by the amount of time spent in a complex environment when

compared to time spent in a simple (control) environment. To my knowledge, no previous studies have examined effects of time in a captive environment in combination with added environmental complexity on foraging behavior. Investigation of zoo animals suggests relocation of individuals to different (novel) captive environments creates a stressful situation for individuals, which can adversely impact behavior and welfare (Dufour et al., 2011). In addition, relocation studies have shown that exposure to differing environments over a short period of time (less than one month) can promote stress in individuals (Molony et al., 2006). Therefore, I predict that individuals that are held for greater than 1.5 months in a simple or complex environment will show more differences in foraging behavior than individuals held for less than 1.5 months in these environments. Foraging differences between individuals in different environments would indicate that not only the structural environment affects behavior, but time in the environment affects behavior as well.

Methods

Study species.

The model species for this study was the meadow vole. This species is widely distributed in North America and is typically found in meadows, lowland fields, and grassy marshes close to water systems (Howe et al., 2006, Krebs et al., 1969, Lindroth and Batzli, 1984). Meadow voles feed mainly on grasses, sedges, and herbs but may eat a variety of seeds as well (Lindroth and Batzli, 1984).

They are diurnal and active year round. Breeding typically occurs during the summer months and winter breeding occurs only in the year preceding a population peak (Keller and Krebs, 1970). This is a promiscuous species where females will mate with several males that overlap her territory (Boonstra et al., 1993). Females actively defend territories (approximately 250 m^2), while males are less defensive of territories and overlap female and male territories (Bowers et al., 1996, Madison, 1980). Females can give birth from two to nine pups, with seven being average, depending on weight of female and population density. Mating may be continuous, as females are induced ovulators and often mate immediately after giving birth (Clulow and Mallory, 1970). Gestation is approximately 21 days, and pups are weaned between 14-21 days after birth (Keller and Krebs, 1970).

This species is short lived; life expectancy is six months in the wild but up to two years in captivity (Wolff, 2003). It plays a critical role in the ecosystem, as it constitutes a considerable portion of many predators' diets (Pusenius and Ostfeld, 2002). In addition, they are proficient diggers, creating runways throughout their territory, which not only facilitates soil nutrient cycling but disperses seeds (Howe et al., 2006).

Study area.

My field site (Figure 2) is located on Winnebago County Park property, on the north side of Oshkosh, Wisconsin. It is owned and maintained by the Winnebago County Parks Department and has casual hiking trails through a marshy meadow. The area,

approximately 2.8 hectares, is fragmented into several smaller areas by diverging trails. Each of the areas, although separated by a 1.8 m wide mowed path, is slightly different in vegetative composition. Overall, the vegetation consists of meadow/scrub plants, including several Aster and goldenrod species (Asteraceae spp.), milkweed (*Asclepias*), sneezeweed (Asteraceae spp.), and tag alder (Alnus spp.). Numerous animals inhabit the area, including goshawk (*Accipiter gentilis*), whooping (*Grus americana*) and sandhill (*Grus canadensis*) cranes, Canadian geese (*Branta canadensis*), Butler's garter snakes (*Thamnophis butleri*), American toad (*Bufo americanus*), two species of mice (*Peromyscus maniculatus* and *Zapus hudsonius*), eastern mole (*Scalopus aquaticus*), short-tailed shrew (*Blarina brevicauda*), eastern cottontail (*Sylvilagus floridanus*), bobcat (*Lynx rufus*), and white-tailed deer (*Odocoileus virginianus*) (personal observation of animal or tracks).

Within three sampling plots, grid point locations were established every 10 meters throughout the site as sampling stations. All traps were 7.5 x 9 x 23cm Sherman traps that were set in the late evening and checked for animals in the early morning every day from mid-May to mid-July, 2011, with a week break in mid-June. Traps were baited with a combination of peanut butter and dried rolled oats, made into approximately 1 cm diameter balls. Cotton was placed in each trap for use as nesting material.

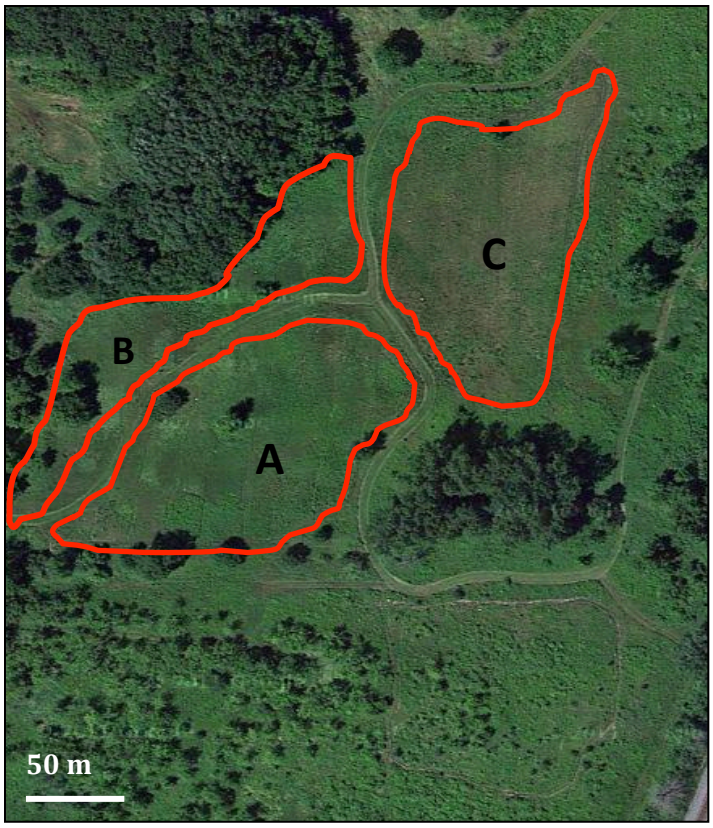

Figure 2: Study area just north of Oshkosh, WI. Three trapping areas are highlighted in white. Trapping points were on a 10 X 10m grid.

Subjects.

Animals were captured and brought into the laboratory between 19 May – 15 June (long group, > 1.5 months) and 29 June – 9 July (short group, < 1.5 months), 2011. Only subadult and adult meadow voles were used in this study and any juveniles caught were released. Age was estimated by weight: ~22-33 g and ≥34 g, for subadult and adult respectively (Krebs et al., 1969, Myers and Krebs, 1971).

Animals brought into captivity were quarantined for two weeks in a room separate from the housing room. The quarantine process involved injecting individuals with a broad-spectrum antiparasite medication, Ivermectin (1:10ml in water dilution, 1ml/0.2kg dosage), and given a full external body rub with a cloth moistened with Adams Flea and Tick mist immediately upon arrival at the laboratory. Weight and sex were recorded for all individuals. Individuals were ear tagged with a metal tag that identified them with a unique number. Pregnant females were permitted to give birth and nurse fully, but the litter was sacrificed once pups were weaned at 21 days.

Experimental groups and captive housing.

Individuals were assigned randomly to either a complex or simple environmental group (Table 1), but consideration was taken to keep sexes equal within each environment. Time of capture determined amount of time spent in assigned environmental cage (long, > 1.5 months and short, < 1.5 months; henceforth referred to as complex long=CL, complex short=CS, simple short=SS, simple long=SL). All individuals were housed individually within one of two cages, except for pregnant females who were housed with their litter until weaning (21 days).

Table 1: Sample size of individuals assigned to environment (simple and complex) and amount of time held in captivity (short, < 1.5 months and long, > 1.5 months). Total of two environments and four time conditions, n = 46.

Captive Lines			
Simple Environment		Complex Environment	
12 ♀ **11**♂		**14** ♀ **9**♂	
Long	Short	Long	Short
5♀ **7**♂	**7**♀ **4**♂	**8**♀ **4**♂	**6**♀ **5**♂

All individuals were housed in the same laboratory room with no windows or additional exposure to the outside. Lighting from fluorescent light bulbs remained on a 14:10h light cycle to simulate summer conditions; on at 0700 and off at 2100 h. Ambient temperature varied between 70-75°F with humidity ranging between 60-80%.

Simple and complex environments were defined by the amount of space and number of unpredictable environmental elements provided in the cage. Simple environmental cages were standard mouse cages, 27.9 x 17.8 x 15.2 cm (according to the Guide for the Care and Use of Laboratory Animals, 1996). Sani-chip bedding and a cotton-fiber nesting square were placed in the cage. Complex environmental cages were 58.4 x 41.4 x 31.4 cm (56 quarts) Sterilite® containers with a removable lid. The lid was modified to allow for airflow by removing the center and replacing it with 0.6cm mesh (~50.8 x 33cm). The containers were also modified to hold one water bottle and a feeding dish immediately adjacent to each other. Two substrates were used to line the complex cage (approximately 7.6 - 10.2 cm of combined substrate): sani-chip bedding

for sanitation purposes and orchard grass hay to simulate natural substrate. In addition, individuals within the complex environmental cages were given a weekly rotation of seven novel objects (Table 2): wheel, curved polyvinyl chloride (PVC) tube, colored chew sticks, wood chew sticks attached vertically within the cage, three small rocks, a noisy "ferret" ball, and a plexiglass barrier with one entrance/exit point. During the first week after being brought into the laboratory, individuals were not given a novel object because the environmental cage was itself novel. Several of the novel objects used needed to be given a second time (after one full rotation of all objects) to individuals assigned to the CL environment, because the number of weeks in captivity outnumbered the amount of novel objects chosen for use. The random assignment of objects provided an element of unpredictability and therefore did not negate the novelty of those objects when placed a second time into the complex cage. All objects were provided as additional complex elements to the caged environment to motivate naturalistic behaviors.

Rat chow pellets (Tekland #8604) and water were given *ad libidum* in both environments. Sunflower seeds were given to supplement their diet (~10 seeds per individual) and were provided three times a week.

Table 2: Significance of novel objects selected for use in cages to simulate a natural environment and behaviors.

Object(s)	Significance
Rodent wheel (purple, pink, blue) (~14cm diameter)	Activity: provides opportunity for exercising and release of excess energy
6.4cm diameter white PVC tube (curved, ~12.7cm)	Environmental stimuli: tunnel effect or cover
Colored (blue, green, yellow, pink) chew sticks (~2 ½ x 2 x 1/2cm)	Activity: provides opportunity to chew and gnaw to keep teeth from overgrowing
Wood chew sticks attached vertically from cage floor (~10.2cm, ~1cm diameter)	Environmental stimuli: vertical structure to stimulate upright vegetation
3 smooth reddish/brown and grey rocks with rounded edges, flat bottom and top (2.5-3.8cm)	Environmental stimuli: natural substrate
Noisy ferret ball (multi-colored: red/yellow, green/red, green/yellow)- loose bell inside semi-permeable plastic ball (~7.6cm diameter)	Activity: a moveable object to create a novel noise and provide opportunity to gnaw
Plexiglass barrier with PVC tunnel (two pieces ~16.5 x 17.8cm attached at ~135° angle)	Activity: opportunity to express spatial ability and problem solving, limits access to food and water by a one entrance/exit point.

All cages were fully cleaned once a week and all individuals were weighed on those days to monitor weight gain or loss. Additional cleaning was performed on a second day if needed. Individuals in simple cages were placed into a completely new cage that was identical to their previous cage. In complex cages, the substrate was completely removed while individuals were held in the weighing container. If females

had pups, the nests were retained and transferred to the new cage to minimize stress and cannibalism.

Behavioral testing.

All individuals were habituated to the testing arena for one hour four days prior to testing to minimize exploration of arena due to novelty and emphasize exploration for food when tested for foraging response. The testing arena was a 0.8 x 1.1 m black plastic bin with 20.3 cm high walls. Each arena had sani-chip lining, and a plexiglass lid was placed on top to eliminate possible escape during testing. Six empty food dishes (constructed from PVC tube caps, 5.1 cm diameter) were placed in a circular pattern within the center of the arena (Figure 3). After one hour, animals were returned to their original cages, and the sani-chip from the arena was placed into a plastic bag to be used during the behavioral test. The purpose of reusing the sani-chip for each individual was again to minimize novelty of the arena environment by providing the individual with familiar odors.

Individuals were food-deprived in their cages for four hours prior to testing to motivate food exploration; water was provided *ad libidum*. Subjects were tested individually. The testing arena was placed behind a curtain to hide the video camera and observer. Three fluorescent lights were placed around the arena to ensure that behaviors were seen clearly in the arena and minimize bias for one side of the arena vs. another. Prior to placing an individual into the arena, three dishes were randomly

selected to contain a 0.5 x 1 cm piece of Macintosh apple. Pilot studies showed that captive voles readily consume apple pieces. Individuals were transferred from their cages to the arena using a small square Tupperware container. Subjects were always placed into the arena in the same corner (Figure 3).

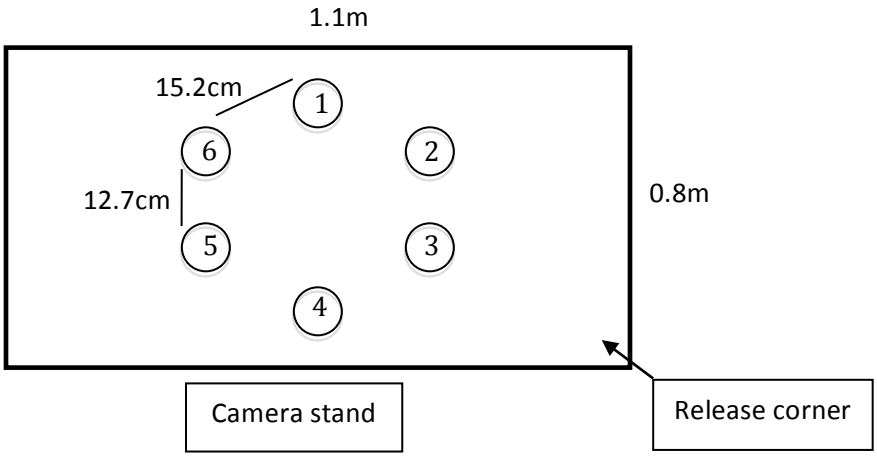

Figure 3: Diagram of testing arena with six possible food dishes. Number in circles indicates number assigned to dish for random placement of food item. Measurements indicate length of arena walls and distance between dishes.

Each test was recorded with a Sony Handycam (Model DCR-SR68) placed on a tripod behind the curtain. Behavioral testing ran for 15 minutes, and the timer began once the animal was released into the arena. Once testing was completed, animals were placed back into their original cages and immediately given rat chow pellets and sunflower seeds.

Analysis.

Video analysis of behavior.

Behavioral tests were video recorded and all videos were transferred to a MacBook Pro OS-X computer. All instances of *a priori* selected behaviors that were observed during trials were recorded using *JWatcher* v1.0 (Blumstein and Daniel, 2007). This program analyzes both duration of behaviors and number of instantaneous events, whose results can be imported into the open source statistical analysis program R (v 2.11.0, RDC Team, 2011). For each test nine parameters were measured (Table 3). All-occurrence (duration of behaviors) and instantaneous (behavior expressed at 5-second intervals) sampling were recorded for each animal during their trial (Altmann, 1974).

Behaviors selected to measure differences in foraging behavior indicated decision-making by voles to locate, harvest, and consume food items. Frequency of consuming, grooming, sedentary, and ambulating would indicate how many times a behavior was observed during a foraging test. Duration (as expressed by proportion of test time) of consumption, time spent at apple dish but not eating (including returning to empty apple dish), and time spent away from all dishes indicate motivation to forage and optimal foraging decision making. Number of apple dishes visited versus non-apple dishes visited shows decision-making of individuals posed with two "patch" quality options; greater interest in apple dishes was assumed to be optimal while greater interest in non-apple dishes is assumed to be sub-optimal.

Table 3: Ethogram of behaviors used to analyze between environments (simple and complex) and time (long and short). All-occurrence (duration of behaviors) and instantaneous (behavior expressed at 5-second intervals) sampling were recorded for each animal during their trial (Altmann, 1974).

Behavior	**Definition**
Events- Instantaneous	**Frequency of a specific behavior expressed**
Consumption	Process of in taking food item, haunches on ground with forepaws holding food to mouth, chewing and ingesting.
Grooming	Repeated movement of head toward distal body parts and extension of tongue to fur, licking, or repeated rapid movement of forearms on proximal and distal body parts, brushing fur.
Ambulate	The process of actively moving forward from a location through movement, alternate extension of fore and hindlimbs (Hansen, 2000), either slowly or quickly.
Sedentary	No movement in any direction, either standing on all four limbs or sitting with rear end on ground with forelimbs straight or trunk of body on ground with hindlimbs tucked under body and forelimbs lying under body.
Visits to apple dishes	Stopping at a dish that contained a food piece for > 1 second.
Visits to non-apple dishes	Stopping at a dish that did not contain food for > 1 second.
States- All occurrence	**Durations of specific behavior expressed**
Consumption	Process of in taking food item, haunches on ground with forehands holding food to mouth, chewing and ingesting.
No consumption at an apple dish	Stopping at an apple dish (whether apple previously consumed or still present) but not handling or ingesting food piece, for > 1 second.
Away from any of the six dishes	Any behavior, previously described, expressed > 3cm distance from any of the six dishes.

Statistical analysis.

All analyses were conducted in the open source statistical program R (v.2.11.0, RDC Team, 2011). The Wilcoxon signed-ranks test was used to evaluate the differences between two samples: simple and complex environment. The Kruskal-Wallis test was used to evaluate the differences across times spent in each environment (SS, SL, CS, and CL). Additionally the Wilcoxon test was used to compare responses between sex and time periods (short vs. long) in environment. Non-parametric statistical testing was used because my behavioral variables were not normally distributed (Runyon and Haber, 1984). To account for multiple comparisons, tests examining foraging behaviors (eating, grooming, ambulate, and sedentary) were compared against an adjusted α of 0.013, number of visits to dishes were considered significantly different at $\alpha = 0.05$, and proportion of behaviors expressed during the 15-minute trial were considered significant at $\alpha = 0.017$.

Results

Forty-six meadow voles (Table 1) that had been housed in a simple or complex captive environment were tested for specific behavioral variables (Table 3) when presented with six dishes, three of which held an apple piece. Behaviors were grouped into two categories to be analyzed: duration of behaviors observed (described as proportion of time) and frequency of behaviors expressed every five seconds, during a

15-minute trial. Resulting P values for differences in median number and proportion of behaviors observed are presented in Tables 4, 5, and 6 for individuals from SL, SS, CL and CS environments. Despite the lack of differences in foraging behavior between environments, time spent in environments had a greater number of significant differences in behaviors observed.

There was no difference in the frequencies of eating, grooming, ambulating, or being sedentary between individuals from a simple or complex environment or between times spent in those environments. In addition, neither environment nor time spent in environment had any effect on the number of visits to non-apple dishes or apple dishes.

Table 4: P-values presented for differences in frequency of behaviors expressed between individuals and sexes (female and male) in environments (simple, complex) and time spent in environment (SS, SL, CS, CL). Refer to Table 1 for sample sizes. P-value for frequency of behaviors were considered significant at $\alpha = 0.013$, and visits to dishes was considered significant at $\alpha = 0.05$.

	Eating	**Grooming**	**Ambulate**	**Sedentary**	**Visits to apple dishes**	**Visits to non-apple dishes**
Environment						
Individuals	0.82	0.72	0.97	0.83	0.62	0.42
Females	0.70	0.21	0.92	0.27	0.19	0.44
Males	0.89	0.26	0.77	0.28	0.39	0.88
Time						
Individuals	0.65	0.02	0.89	0.33	0.17	0.07
Females	0.96	0.02	0.66	0.55	0.14	0.14
Males	0.22	0.34	0.89	0.34	0.28	0.42

Proportion of time spent consuming, visiting an apple dish but not eating, and being away from any of the six dishes were analyzed. I measured differences in duration of activities in the arena between individuals from different environmental and time groups. There were no differences between individuals from simple and complex environments or between females and males in those environments (Table 5). To search for differences in proportion of time spent at an apple dish but not eating, individuals that did not visit any apple dish were removed from the data set (from simple n = 5 and complex n = 4). Remaining individuals had been observed returning to empty apple dishes (apple piece previously consumed) and visiting apple dishes but not eating apple piece. Comparison of median proportion of time spent on an activity between time groups in simple and complex environments showed a significant difference amongst individuals at an apple dish but not eating ($\chi^2 = 12.55$, df = 3, p = 0.01, $\alpha = 0.02$) (Figure 4A, Table 5). More specifically, only individuals housed in a SL and SS environment showed a significant difference in proportion of time spent at an apple dish but not eating (Figure 4B, Table 6). Individuals included in analysis of proportion of time spent at apple dish but not eating still showed a significant difference between time groups. There was a significant difference between individuals from time groups that both visited apple dishes but did not eat and visited empty apple dishes ($\chi^2 = 12.01$, df = 3, p = 0.01, $\alpha = 0.02$; n = 30).

Table 5: P-values of difference in proportion of behaviors expressed over 15 minutes between individuals and sexes (female and male) in environments (simple and complex) and time spent in environments (SS, SL, CS, CL). Refer to Table 1 for sample sizes.

	Proportion spent eating	Proportion at apple dish but not eating [+]	Proportion spent away from any of six dishes
Environment			
Individuals	0.96	0.40	0.59
Females	0.68	0.21	0.15
Males	0.66	0.62	0.38
Time			
Individuals	0.75	**0.01***	0.10
Females	0.46	0.10	0.34
Males	0.54	0.07	0.08

*Significance at $p < 0.017$.

[+]Includes only animals that visited apple dishes; either returned to an empty apple dish because apple piece previously consumed or visited apple dish but did not eat apple piece (23 individuals only returned to empty apple dishes, 7 individuals returned to empty apple dishes and visited apple dishes without eating, 7 individuals just visited apple dishes without eating.)

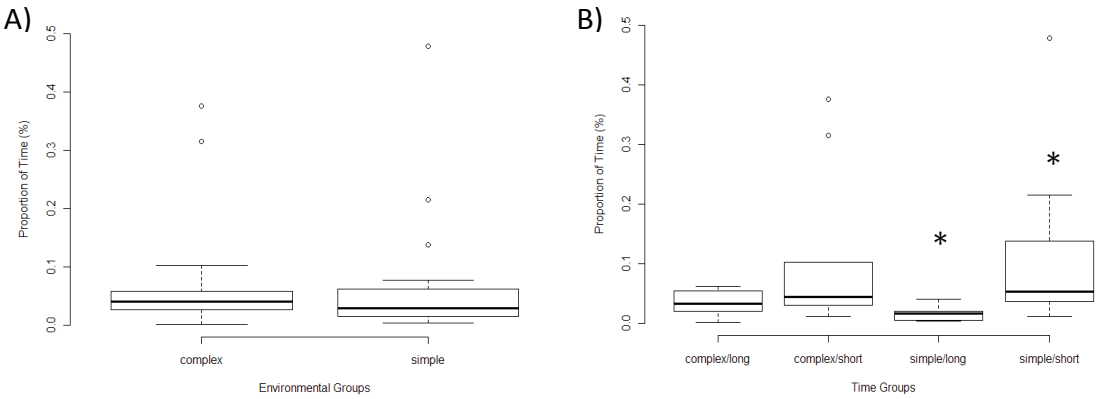

Figure 4: A) Proportion of time spent at an apple dish but not eating between environments (n = 18 for simple and n = 19 for complex environment; excluded n=5 from simple and n = 4 complex environments). B) Proportion of time spent at an apple dish but not eating between times spent in environment (CL n = 10, CS n = 9, SL n = 8, and SS n = 10). Only individuals that visited apple dishes were included. Significant difference, $p < 0.01$, between groups indicated by

asterisks.

Analysis of sex and time differences within simple and complex environments did not show significant differences in number of foraging behaviors observed (Table 6). Females performed more ambulatory behavior than males after being housed in a complex environment. Interestingly, there was a significant difference in number of visits to apple dishes between females and males housed in a complex environment ($W = 97$, $p < 0.05$) (Table 6): females visited more apple dishes than males (Figure 6). There were, however, no significant differences in proportion of time spent eating ($p > 0.05$) or not eating at apple dishes ($p > 0.05$) between females and males in a complex environment. In comparison, females and males housed in a simple environment did not show any differences in foraging behaviors during trials (Table 6). While sex differences were only shown between individuals held in a complex environment and not in the simple environment, foraging behaviors did not differ between males from either environment or females between either environment (Table 4 and 5).

Table 6: Difference between sexes (female vs. male) and time (short vs. long) in simple and complex environments independently. Refer to Table 1 for sample sizes.

Variables	Sex/simple environment	Sex/complex environment	Time/simple environment	Time/complex environment
Number of observations				
Foraging				
Eating	0.18	0.47	0.31	0.81
Grooming	0.58	0.26	0.02	0.04
Ambulate	0.06	0.02	0.62	0.74
Sedentary	0.04	0.95	0.07	0.58
Dishes visited				
Food dishes	0.88	**0.03***	0.06	0.39
Non food dishes	0.38	0.07	**0.03***	0.37
Duration of observations				
Proportion spent eating	0.95	0.35	0.64	0.33
Proportion at apple dish but not eating[¤]	0.70	0.50	**0.00†**	0.28
Proportion spent away from dishes	0.98	0.02	0.02	0.88

A significance level of: $\alpha < 0.013$ for four behaviors (eating, grooming, ambulate, and sedentary), $\alpha < 0.05$* for number of visits to dishes, and $\alpha < 0.017$† for proportion of behaviors observed.
[¤]Only included a subset of individuals; simple environment n = 18, complex environment n = 19; excluded individuals that did not visit any of the six dishes.

Approximately the same number of males and females in each environment (simple and complex) did not eat any apple pieces during the foraging test. There was no difference between individuals from the simple and complex environment and number of apple pieces consumed. However, a greater percentage of individuals from the SS (45%) and the CL (50%) environment ate all three apple pieces than individuals from the SL (17%) and the CL (9%) environment (Table 7).

Table 7: Percent of individuals housed in different environments (simple and complex) for housed for a short and long period of time that ate 0, 1, 2, 3 apple pieces during a 15 minute foraging test. Refer to Table 1 for sample size.

Number of apple pieces eaten	Simple (%)		Complex (%)	
	Short (SS)	Long (SL)	Short (CS)	Long (CL)
0	18	42	45	33
1	9	25	18	8
2	27	17	27	8
3	45	17	9	50

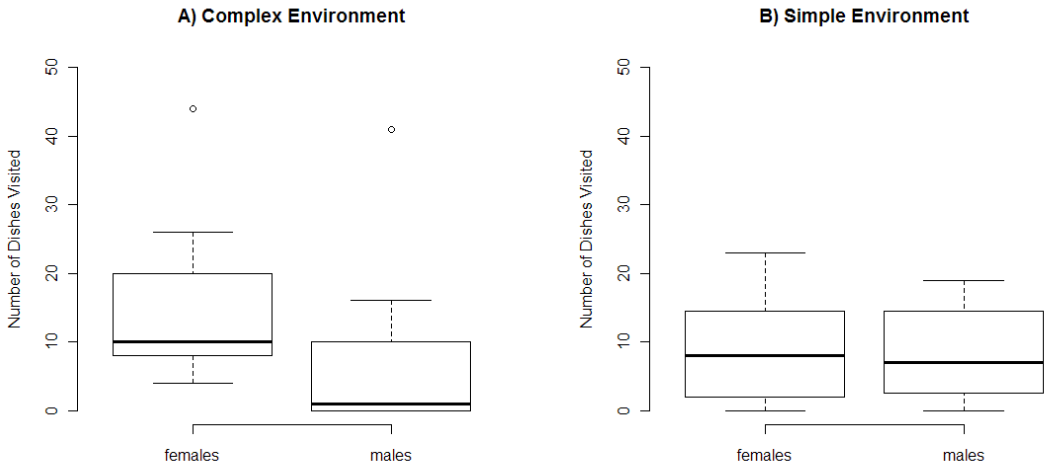

Figure 5: Number of visits to apple dishes by females and males in the A) complex environment ($p = 0.034$, $\alpha = 0.05$) and B) simple environment ($p = 0.88$, $\alpha = 0.05$). Refer to Table 1 for sample size.

Individuals housed for short versus long time in a simple environment showed a

significant difference in number of visits to non-apple dishes ($W = 29.5$, $p < 0.03$, $\alpha = 0.05$) (Table 6). Individuals housed in a SL environment visited non-apple dishes significantly less than those housed for a short time (Figure 6).

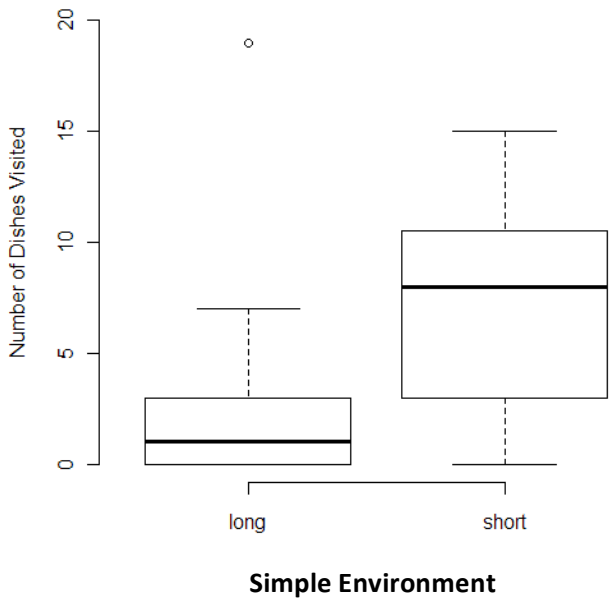

Figure 6: Number of visits to non-apple dishes, between individuals housed in a simple environment for a long and short period of time ($p = 0.025$, $\alpha = 0.05$). Refer to Table 1 for sample size.

Discussion

Individuals from simple and complex environments and housed for both a short and long time did not express significant differences in frequency and duration of foraging behaviors. This suggests that providing complexity within the captive environment for adult meadow voles does not affect behaviors more than a simple

environment. Studies have suggested that complexity or environmental enrichment is more beneficial during developmental stages and has more of an effect on behaviors than on adults who have already developed behavioral repertoires (Kelley et al., 2005, Miller et al., 1990, Odberg, 1987, Stoinski et al., 2003, Sundstrom and Johnsson, 2001, Vargas and Anderson, 1999). Cooper et al. (1996) showed that there are limitations to the effectiveness of environmental enrichment. In their study, older individuals of bank voles (*Clethrionomys glareolus*) exhibited stereotypic behaviors in both a barren and a complex (although slightly fewer individuals) environment, yet no juveniles exhibited stereotypic behaviors in a complex environment (Cooper et al., 1996). This suggests that younger individuals during their developmental stage benefit more from complexity than do developed adults. However, for adult individuals, added complexity within the captive environment has been suggested to minimize stereotypical behaviors (Watters and Meehan, 2007) and maintain well-being (McPhee and Carlstead, 2010) almost immediately.

Previous studies have shown the importance of space in the captive environment for the development and maintenance of natural skills (Novak et al., 2004, Prescott and Buchanan-Smith, 2004). However, Odberg (1987) showed that in bank voles, development of stereotypies was more dependent on enrichment than cage size. In my study, meadow voles were exposed to a large cage and environmental enrichment and yet minimal significant differences in foraging behavior were observed between

groups. This may have also been a result of using adult subjects rather than juveniles. However, previous investigation of the influence of cage size in combination with environmental complexity on behavior of cottontop marmosets (*Saguinus oedipus*) (Kitchen and Martin, 1996) did cause increased activity and enhanced welfare. Future study should examine activity within different captive environmental conditions in comparison with results from behavioral testing.

Individuals from the SL environment visited fewer apple dishes than individuals from the SS environment. This would support the idea that individuals who are housed in a simple environment (lacking opportunity to express foraging skills) for greater than 1.5 months are less likely to explore environments and seek out food resources than those housed for less time. Lack of activity can indicate poor welfare in captive individuals, which may affect physical health, potential use in research, or reintroduction efforts (Baumans, 2011).

Individuals from the CL, CS, and SS time groups expressed the same proportion of time spent at an apple dish not eating, but individuals from the SL environment spent significantly less time at an apple dish not eating. The opportunity to cache food in the complex environment compared to the simple environment may explain the response differences to predictable food availability. When individuals in the complex cage cached their food pellets, thereby emptying their food dish, the dish was replenished. Animals in a complex environment were able to empty their food dish without having to

consume all the pellets, and caching pellets allowed for individuals to express foraging skills to relocate their caches (Bassett and Buchanan-Smith, 2007). Individuals from a SL environment spent less time visiting any dish, and a greater percentage did not eat any apple piece. However, there were no differences in frequency and duration of eating behavior between individuals who did eat. This would suggest that being housed in a SL environment affects an individual's motivation to locate food resources but not consumption of food resources. It is highly unlikely that one group over another could have been sufficiently impacted by the food deprivation period to decrease that individual's want or ability to locate and consume food. All animals were randomly selected for testing, and all were treated exactly the same. Therefore, it is reasonable to assume that all results obtained are because of environmental differences in captivity.

Interestingly, foraging differences were inconsistent between sexes in simple and complex environments. These differences highlight how a simple or complex environment might affect females and males independently. Females and males require different amounts of resources and time spent accumulating those resources based on reproductive costs (Ginnett and Demment, 1997, Low, 2000). Females have greater energy requirements to meet the energy costs of gestation and lactation (Key and Ross, 1999). As energetic demands increase in females they will tend to feed longer than males and prefer higher caloric foods (Gittleman and Thompson, 1988). Males are considered to be less cautious than females in the presence of risk, such as a predator

(Low, 2000), because their reproductive success is dependent on finding and acquiring a female. Behavioral analysis suggested that females from a complex environment visited apple dishes significantly more than males, yet both spent the same proportion of time eating and returning to empty apple dishes. When compared to no sex differences in the simple environment[a], the complex environment promotes sex-specific behaviors, which are important for future reproductive success. Studies have shown that females who have altered parental care behaviors (including proper maintenance of body during pregnancy and nursing) can neglect offspring, which can lead to disadvantageous changes in development of critical behaviors (Archer and Blackman, 1971, Moore, 1984).

Behavioral testing was conducted in a predator-free environment, although environmental structure could be a cue of predation risk, and the absence of any "cover" within my testing arena could have affected an individual's behaviors during the trial (Arcis and Desor, 2003). The methods used in my study limit my interpretation of the results to only distinct behavioral differences as a result of temporary housing in simple and complex environments. I cannot firmly conclude whether significant differences of expressed behaviors between environments promoted optimal foraging strategies. All individuals were exposed to the foraging arena for one hour four days prior to testing, and during this habituation trial no food was placed into any of the dishes, but individuals could have become familiarized with the "patches" (dishes).

[a] Six females housed in the simple environment and eight females housed in the complex environment delivered a litter in captivity soon after being captured in the field. All litters had been weaned at the latest two weeks prior to testing.

During the foraging test, randomized "patches" contained a food item, but individuals had no prior knowledge of "patch" quality (good = food, bad = no food). However, the argument might arise that individuals were given prior knowledge of patch quality (i.e. poor quality) during the habituation trial, and individuals from the SL environment may have reacted appropriately to that knowledge. By contrast, it can also be argued that individuals from the three other environments, SS, CS, and CL, also responded appropriately to the element of unpredictability within an environment, which is a main component of the wild environment. Therefore, it is more likely that these individuals responded more appropriately to the foraging test because of exposure to the unpredictable rotation of objects (in CS and CL) and reduced time in captivity (in SS) minimized habituation to predictability.

Although baseline behaviors were not assessed when individuals were brought into captivity, I assume that all individuals expressed a similarly natural range of behaviors because they had been captured from the wild at the same field site and therefore exposed to the same conditions. Individuals assigned to time groups in both captive environments were captured during different time intervals over the summer (early to mid summer and mid to late summer). Therefore, any effects of time spent in captivity may be a result of differences in body maintenance and experience in the wild

immediately after snow melt versus later in the summer. Individuals housed in a complex environment for a long and short period, however, do not exhibit the same behavioral differences compared to individuals housed in a simple environment for a long and short period of time. This may therefore discount any seasonal effects.

While I cannot speculate as to how much the captive environments changed an individual's initial foraging behaviors I can presume that any behavioral differences that did occur were due to the experience within the environment in which an individual was housed. For these reasons, this study would suggest that time spent in a captive environment effects foraging behavior more than complexity alone. Still, complexity within the environment reduces effects of time, such as predictability and habituation, more than in an environment lacking complexity.

Last, the overwhelming number of behaviors that were marginally significant between groups indicates a serious sample size issue. This highlights the importance of obtaining a large enough sample size of test subjects and can be difficult to do when working with threatened or endangered species whose population size are very low. Therefore, the use of model organisms to examine behavior is a preferred alternative. In this study a model organism was used, but time and available space limited the number of subjects that could be tested.

Conclusions

Understanding how behaviors change in varying environments is critical for providing optimal captive environments that maintain natural behaviors to promote healthy well-being. This will also lead to increased success of captive-reared and captive-housed individuals upon release into the wild.

The limited behavioral change between individuals in environmental conditions suggests that the captive environment may not affect behaviors of adults as dramatically as individuals that have not developed a foraging repertoire. This may be because adults have already developed skills in the wild, and the time that they were housed in captivity was insufficient to influence foraging behaviors. The use of relocation as a management tool can only be successful if wild behaviors are maintained during time in captivity (Beissinger, 1997, Rabin, 2003). Therefore, time spent in captivity should most likely take precedent over captive environmental conditions when using older individuals during relocations.

This study also highlights that, while understanding how complexity in captivity affects the maintenance of naturalistic behaviors, management focus should also be placed on how complexity affects behaviors fundamental to females and males. Therefore, the captive environment should be tailored to females and males independently to increase breeding success and persistence of healthy future generations.

Chapter III

Effects of Environmental Complexity and Time Spent in Captivity on Survival of Meadow Voles in the Wild

Introduction

As species struggle to maintain their share of the planet, management agencies are increasingly using captive breeding and translocations to preserve species diversity. Since reintroduction efforts began in 1907 with the American Bison (*Bison bison*) in Oklahoma (Kleiman, 1989), there have been marginal increases in successful re-establishment of self-sustaining populations in the wild (Beck et al., 2002, Fischer and Lindenmayer, 2000). There are several reasons why reintroductions have been difficult to implement across species: 1) high costs, 2) logistical difficulties, and 3) shortage of suitable habitats (Kleiman, 1989, Kleiman et al., 1994). Reintroduction becomes more difficult with the use of captive-bred individuals (Jule et al., 2008, Mathews et al., 2005) because individuals are often ill-equipped to respond appropriately to the wild environment when released (Beck et al., 2002). Individuals lack knowledge of predators (Griffin et al., 2000), natural foods and collection techniques (Stoinski and Beck, 2004), social interactions (Glatston et al., 1984, Snyder et al., 1996), parental care (Hannah and Brotman, 1990, Moore, 1984), and mating (Yamada and Durrant, 1989), and they do not possess the appropriate mechanisms to overcome diseases (Cunningham, 1996) and

environmental unpredictability. According to Snyder et al. (1996), captive breeding should be a last resort for species conservation because the lack of current understanding of how genetics and behaviors are affected and how to prevent losing critical traits. However, when the population size of a species becomes too low in the wild, a captive-breeding program to supplement and reinforce the species' population becomes necessary [e.g. black-footed ferret *Mustela nigripes* (Biggins et al., 1998), red wolf *Canis rufus* (Phillips and Parker, 1988), partula snail *Partula taeniata* (Pearce-Kelly et al., 1995), Vancouver marmots *Marmota vancouverensis* (Aaltonen et al., 2009)]. At this point, however, there is limited room for error when implementing techniques and procedures.

The main cause for individual loss upon release into the wild is that over time in captivity, life history and behavioral traits change so that they do not resemble those of wild conspecifics. Darwin (1868) hypothesized that the environment to which an individual is exposed alters its morphological, physical, and behavioral traits. Because the captive environment is dramatically different from the wild, we would expect it to significantly decrease an individual's opportunity to develop and maintain appropriate behaviors for the wild. Many challenges that are present in the wild are absent in captivity, and without these experiences, an individual's behavioral repertoire may be permanently altered (Britt, 1998). Numerous studies conducted on captivity's effects on reintroduced individuals have provided strong support that the captive environment

promotes deficiencies that can be fatal in the wild (Biggin et al., 1999, Kleiman et al., 1990, Miller et al., 1994, Shier and Owings, 2006, Stoinski and Beck, 2004). Until recently, there have been few studies that rigorously examine effects of captivity on critical behavioral traits and survival in the wild (Mathews et al., 2005, Seddon et al., 2007, Sutherland, 1998). However, these studies are crucial for understanding factors that would inhibit behavioral deterioration and failure of reintroductions. Much emphasis in the conservation field has been on failures, but there have been many successful releases. Success depends on producing a surviving and self-sustaining population by providing a suitable habitat, effective techniques to prepare the animal, post-release training and monitoring, habitat protection and management, and public education (Kleiman, 1989). To increase the probability of release success, more hypothesis-driven research must be conducted. Through this research, we can develop broad concepts that may be applicable across species. For this study, I focused on effective techniques that may prepare animals in captivity to successfully adjust to the wild environment when released.

Previous studies have shown that adding complexity to the captive environment can increase survival by maintaining appropriate behavioral responses in mating, foraging, and predator avoidance (Sanz and Grajal, 1998, and White et al., 2005). Complexity may also provide learning experiences (for juvenile individuals) that are essential for proper development similar to that experienced in the wild. To investigate

how the captive environment affects survival in the wild, I examined the effects of environmental complexity in captivity on survival in the wild of a model species, the meadow vole (*Microtus pennsylvanicus)*.

For this study, I focused on providing a complex environment that may stimulate and maintain foraging behavior because meadow voles spend a majority of time searching, locating, and caching food items. Its short lifespan also allows for a short but meaningful monitoring period of survival in the wild.

My objective in this study was to determine whether complexity within the captive environment maintains natural survival rates when animals are released into the wild. I predicted that individuals housed in a complex environment would show survival rates similar to wild individuals, but individuals housed in a simple environment would have a lower survival probability. I also examined how time spent in a complex environment affects survivorship in the wild. Few studies have examined how time affects survival, but relocation of declining species or species of conflict can be a cheaper alternative to conservation management (Griffith et al., 1989). Relocation involves housing individuals in captivity temporarily, but often the stress of being handled and novelty of changing environments can cause efforts to be unsuccessful (Molony et al., 2005). Therefore, it is important to understand how temporary housing in captivity and whether complexity during this time affects survival of relocated individuals. I predicted that voles held in captivity for less than 1.5 months, independent

of environment, would show similar survival rates as wild voles (control). I also predicted that being housed in a complex environment would result in similar survival rates as individuals that are housed for less than 1.5 months and the wild (control).

Methods

Study species.

Refer to Chapter II for biology of meadow voles.

Study area.

Grid points were set up every 10 meters throughout the field site (refer to Chapter II for detail on field site) for trapping. All traps were 7.5 x 9 x 23 cm Sherman traps that were set in the late evening and checked for animals in the early morning everyday from mid-May to mid-July, 2011 with a week break mid-June. Traps were baited with peanut butter and dried rolled oats, made into approximately 1 cm diameter balls. Cotton was placed in each trap for use as nesting material.

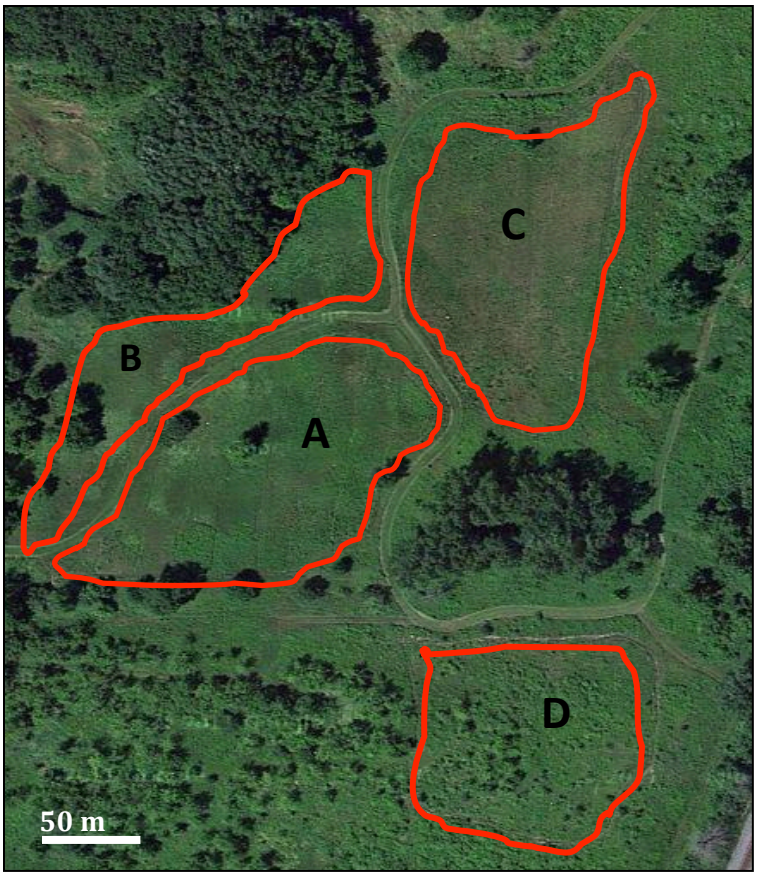

Figure 1: Study area just north of Oshkosh, WI. Four trapping areas are highlighted in white; 10x10 m grid points were set up in each area. An enclosure was not constructed around plot B but the area was used to capture meadow voles throughout the summer. Plots A, C, and D were approximately 1 acre, enclosed and used for releasing the animals as described in the text.

Subjects.

Animals were captured and brought into the laboratory between 19 May -15 June (first group) and 29 June - 9 July (second group), and 5 and 6 August (wild group), 2011. Only subadult/adult meadow voles were used in this study and any captured

juveniles were released. Age was estimated by weight: ~22-33g and ~34+ g, for subadult and adult respectively (Krebs et al., 1969, Myers and Krebs, 1971).

Refer to Chapter II for quarantine process of animals brought into the lab.

Experimental groups and captive housing.

Individuals were assigned randomly to either a complex, simple, or wild environmental group (Table 1). Consideration was taken to keep sexes equal within each environment. Time of capture in the field determined amount of time spent (long, > 1.5 months and short, < 1.5 months) in assigned environmental cage (henceforth referred to as SS = simple short, SL = simple long, CS = complex short, CL = complex long). All experimental individuals (in complex and simple environments) were housed individually within a cage except for pregnant females who were housed with their litter until nursing was completed (21 days). Wild individuals were housed in standard mouse cages with sani-chip bedding, exactly as individuals housed in the simple environment. Individuals assigned to the wild environmental group were housed in captivity for less than 10 days and were handled minimally to reduce any habituation to human interaction and captivity.

Refer to Chapter II for housing of experimental groups (simple and complex environment).

Table 1: Sample size of individuals assigned to environment (simple, complex, and wild) and time held in captivity (short, < 1.5 months and long, > 1.5 months). Total n = 55.

Captive Lines				**Wild Line**
Simple Environment		Complex Environment		Wild Environment
12 ♀ **11**♂		**11** ♀ **8**♂		**5** ♀ **8**♂
Long	Short	Long	Short	< 10 days
5♀ **7**♂	**7**♀ **4**♂	**4**♀ **4**♂	**7**♀ **4**♂	**5** ♀ **8**♂

Reintroduction.

Release.

All subjects were simultaneously released on 17 August 2011 at the study area where originally captured (Figure 1). Three release enclosures, 0.4 hectares, were built to minimize immigration from the study area and resident wild voles were removed to reduce competition with release animals. Individuals were randomly assigned to an enclosure and a grid point within an enclosure (enclosure A n = 18, enclosure C n = 19, enclosure D n = 18). Consideration was taken not to cluster individuals from the same environmental treatment at neighboring grid points within enclosures.

A soft release method was used to minimize stress and immediate loss of animals upon release (Bright and Morris, 1994, Letty et al., 2000). This type of release method involves leaving Sherman traps locked in the open position and baited with an apple slice to provide nutrients and moisture. All traps also had a cotton fiber nesting

square for bedding and were left open in the field for one week before monitoring began.

***Monitoring*.**

Subsequent monitoring of survival began the week following the release. Trapping occurred for three nights on weekends from 25 August until 29 September 2011, but inclement weather prohibited some weekend trapping. Typically trapping occasions would be consistent intervals, but due to inclement weather during Fall of 2011, full three day weekends (intervals) were not possible. Therefore each day that traps were set were considered a single trapping occasion. Traps were not set if temperatures were forecast to drop below 50° F overnight. Traps were baited with a mixture of peanut butter and rolled oats, and a cotton fiber nesting square was included. All traps were checked before 0800 hours to minimize any losses due to food deprivation or overheating in traps. Individuals captured in a trap were immediately weighed, checked for parasites, females were checked for pregnancy signs, and their ID number was recorded. All individuals were released at their point of capture immediately after data were recorded. Trapping was terminated at the end of September due to consistently cold nighttime temperatures.

Data analysis.

Mark-recapture data collected from released voles were analyzed using program MARK (White, Colorado State University, Fort Collins, CO). To analyze parameter

estimates of live recapture data in a closed population, the Cormack-Jolly-Seber (CJS) model (Cormack 1964, Jolly 1965, and Seber 1965) was used. The CJS model follows several assumptions: 1) every marked vole present in the population at time (*i*) has the same probability of recapture (ρ); 2) every marked vole in the population immediately after time (*i*) has the same probability of surviving to time (*i*+1); 3) marks are not lost or missed; 4) all samples are instantaneous, relative to the interval between trapping occasions (*i*) and (*i*+1), and each release is made immediately after the sample (Cooch and White, 2006). Assumptions 3 and 4 were met because each individual was marked with a numbered tag, and animals were released immediately after their identification number was recorded. Assumptions 1 and 2 needed to be tested for lack-of-fit to the data sets that may have been caused by parameter heterogeneity. The main goal was to determine whether apparent survival probabilities were dependent on captive environment type and time spent in captivity, but because some individuals may have escaped detection while they were alive, apparent recapture probabilities also needed to be estimated. Only apparent survival and recapture probabilities could be estimated because both deaths and emigration could not be separately determined.

Time intervals between the 12 trapping sessions (aka encounter sessions) ranged from 1 to 9 days (9, 1, 1, 5, 1, 6, 1, 1, 5, 7, 6) and this inconsistency was manually adjusted for in MARK. Models selected for testing (Table 2) were analogous to hypothesis testing; the most parsimonious model determines whether the null

hypothesis is rejected or accepted. The initial release day was counted as first encounter session. For environment and time spent in environment groups (Table 1), I ran the general model where the group and time spent trapping interaction affected survival and recapture probabilities (e.g. complete, fully-time spent trapping- and group-dependent model); φ (g*t) ρ (g*t), where φ = survival probability and ρ = recapture probability. I also ran all possible subsets of the general model (Table 3). Candidate models were evaluated on the basis of support by comparing Akaike's Information Criterion (AICc) values in MARK (Akaike, 1973, Burnham and Anderson, 2002, White et al., 2005). The "best" fit model was selected based on the lowest AICc value and number of estimated parameters. If multiple competing models received support (ΔAICc) of less than a value of two then the likelihood ratio test was run to determine similarity of nested models and were considered to be indistinguishable if $p > 0.05$ (Simonoff, 2003). All models were selected (Table 3) *a priori* and the lowest AICc value was used to determine the model that best fit my data.

To account for any variation or dispersion in the data, the variance inflation factor, ĉ (Cooch and White, 2006, Lebreton et al., 1992) was calculated. Goodness- of-fit (GOF) and Program RELEASE were run to test the general model's fit and for any underlying heterogeneity, respectively. Program RELEASE performs two tests to understand why a starting model may be rejected: Test 2 (test of independence) and Test 3 (test of heterogeneity), and is a fundamental first step. I used the bootstrap GOF

function with 1000 simulations to find the probability of obtaining the general model's deviance. By using the bootstrapping method, I could determine how closely the general model fit the data ($\hat{c}$ =1, a perfect fit) (Burnham and Anderson, 1998); values of $\hat{c} < 1$ indicate underdispersion and > 1 overdispersion. The $\hat{c}$ was calculated by dividing the general model's deviance (observed deviance) by simulated deviance (expected deviance). This value can then be applied to the remaining models in the set of models considered during the analysis (changing AICc to QAICc). Due to the small sample size and few encounter occasions, I anticipated some underdispersion. If underdispersion occurs ($\hat{c} < 1$), Cooch and White (2006) recommend not adjusting $\hat{c}$ but rather using the default value of 1.00. This helps to ensure conservation in the model selected.

Table 2: Notation for group and time effects on survival (φ) and encounter (ρ) probability parameters in the Cormack-Jolly-Seber (CJS) model of released meadow voles.

Notation	Model Description
Group	Environment: simple, complex, and wild Time: simple short, simple long, complex short, complex long, and wild
Time	Time spent trapping in the field
φ (group*time) / ρ (group*time)	*General model*- both survival and encounter rate are dependent on group and time interaction
φ (group*time) / ρ (.)	Survival rate is dependent on group and time interaction, encounter rate is constant over group and time
φ (.) / ρ (group*time)	Survival rate is constant over group and time, encounter rate is dependent on group and time interaction
φ (time) / ρ (time)	Both survival rate and encounter rate are time dependent
φ (time) / ρ (.)	Survival rate is time dependent, encounter rate is constant over time
φ (.) / ρ (time)	Survival rate is constant over time, encounter rate is time dependent
φ (group) /ρ (group)	Both survival and encounter rate are group dependent
φ (group) / ρ (.)	Survival rate is group dependent, encounter rate is constant over group
φ (.) /ρ (group)	Survival is constant over group, encounter rate is group dependent

Results

A total of 55 meadow voles from captivity and the wild (Table 1) were marked and released into three 0.4 hectare outdoor enclosures. Overall, 58.2% (23/55) of reintroduced voles survived until some point after initial release.

Environmental group effect.

The general model (φ (g*t) ρ (g*t)) was the lowest-ranked model, but because of the sparseness of the data and possibility for lack-of-fit, program RELEASE was run. No underlying heterogeneity was evident in the general model, suggesting that the general model structure was appropriate (Test 2 $\chi^2 = 7.31$, df = 20, p = 0.10; Test 3 $\chi^2 = 1.40$, df = 7, p = 0.10). The GOF test was then run on the general model to determine the value of the variance inflation factor (ĉ) to indicate whether there was any loss of precision, or bias of the estimate (Burnham and Anderson, 2002), from the model's fit to the data. The simulated mean deviance (expected deviance) from the GOF test (1000 simulations) was divided by the general model deviance (observed deviance) to calculate ĉ. The resulting ĉ, 0.7, indicated underdispersion of the data; thus the default ĉ of 1.00 was used. Based on the criterion of a ΔAICc value < 2, three models had an AICc that was not significantly different from the best-fit model (Table 3). These four models were considered indistinguishable (LRT φ (.) ρ (.), φ (g) ρ (g) = 0.05), and the fully group-dependent model (φ (g) ρ (g)) was selected as the best fit model because of the greater number of estimated parameters (6) used to evaluate the model.

Table 3: Model selection for apparent survival (φ) and recapture (ρ) of mark-recapture data from environment groups released into three enclosures.

Model	AICc	ΔAICc	AICc Weights	Number Parameters
φ (.) ρ (.)	**505.99**	**0**	**0.366**	**2**
φ (g) ρ (.)	**506.95**	**0.96**	**0.226**	**4**
φ (.) ρ (g)	**507.06**	**1.07**	**0.215**	**4**
φ (g) ρ (g)	**507.70**	**1.71**	**0.156**	**6**
φ (t) ρ (.)	512.13	6.14	0.017	12
φ (.) ρ (t)	513.37	7.38	0.009	12
φ (t) ρ (g)	514.02	8.03	0.007	14
φ (g) ρ (t)	514.76	8.77	0.005	14
φ (t) ρ (t)	521.02	15.03	0	21
φ (.) ρ (g*t)	537.52	31.52	0	34
φ (g) ρ (g*t)	539.97	33.98	0	36
φ (g*t) ρ (.)	550.00	44.01	0	34
φ (t) ρ (g*t)	550.29	44.30	0	43
φ (g*t) ρ (g)	553.11	47.12	0	36
φ (g*t) ρ (t)	562.78	56.79	0	43
φ (g*t) ρ (g*t)	604.67	98.68	0	63

Models with a ΔAICc value < 2 were considered "best" fit models, indicated in bold
Model notation: φ survival, ρ recapture, (g) environmental group, (t) time spent trapping, (.) no effect

The estimated probabilities for survival and recapture for all three environment groups (simple, complex, wild) were calculated in MARK. The survival and recapture estimates for individuals from the simple environment were 0.959 (SE = 0.01) and 0.73 (SE = 0.05). For individuals from the complex environment, survival and recapture estimates were 0.97 (SE = 0.01) and 0.72 (SE = 0.05). Lastly, for individuals from the wild environment survival and recapture estimates were 0.97 (SE = 0.01) and 0.59 (SE = 0.06). Survival and recapture probability estimates for all three environment groups

were not significantly different between environment (Kruskal- Wallis df = 2, $p_{survival;\ recapture} = 0.37$). However, trends suggest individuals from the complex environment had survival probabilities similar to individuals from the wild environment (Figure 2) and individuals from the simple and complex environments showed higher recapture probabilities than wild individuals (Figure 2).

Table 4: Mean estimates of apparent survival and recapture probabilities with associate standard error (SE) and 95% confidence intervals (CI) for environment groups over 12 encounter occasions. Survival and recapture estimates produced from group only Cormack-Jolly-Seber model (φ(g)/ρ(g)) in program MARK.

Group	N[a]	Estimate	SE	95% CI
Apparent Survival				
Simple	23	0.95	0.01	0.92- 0.97
Complex	19	0.97	0.01	0.95- 0.98
Wild	13	0.97	0.01	0.94- 0.99
Apparent Recapture				
Simple	23	0.73	0.05	0.62- 0.82
Complex	19	0.72	0.05	0.62- 0.80
Wild	13	0.59	0.06	0.47- 0.71

[a]Sample size (N) represents number of voles marked and initially released

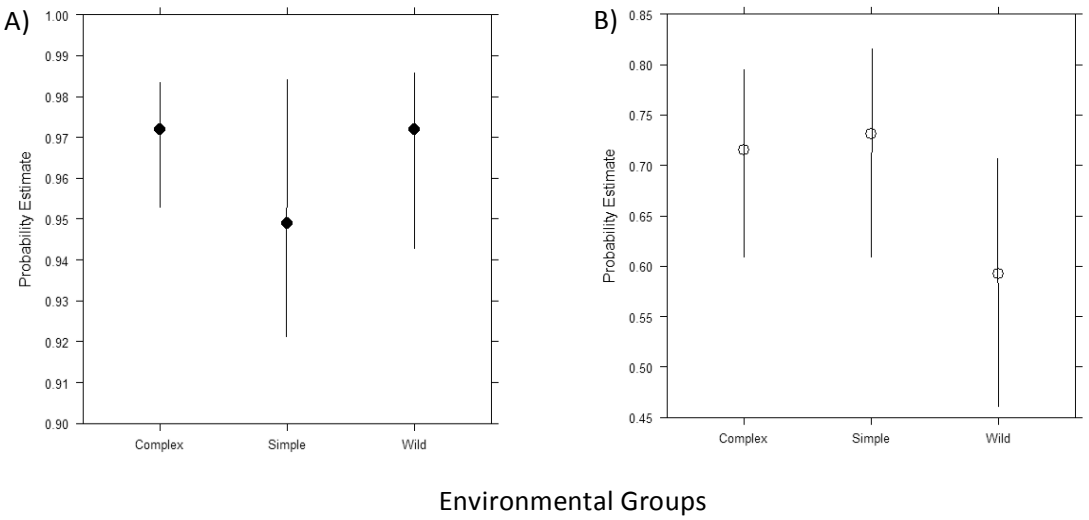

Figure 2: Mean estimates (and 95%CI) for apparent A) survival, black circles, and B) recapture probability, clear circles, of environment groups (simple, complex, and wild).

Time spent in captive environment.

The general model (φ (g*t) ρ (g*t)) with a full group and time interaction was also used to evaluate mark-recapture data of individuals held in captive environments for less than and greater than 1.5 months in comparison to wild individuals. This general model was ranked lowest when all other candidate models were run together (Table 6). Program RELEASE results showed that all time groups were homogenous (Test 2 χ^2 = 5.99, df = 22, p = 0.10; Test 3 χ^2 = 1.14, df = 3, p = 0.77) for parameters in survival and recapture probabilities and no changes to general model structure were needed. The calculated ĉ, 0.69, indicated a measure of underdispersion of the data and a default

ĉ = 1.00 was used to assess models. A fully group-dependent model resulted in greatest support (ΔAICc = 0; Table 7), with all 10 estimated parameters. In addition, the candidate and nested model φ (.) ρ (g) was also well supported (ΔAICc = 0.69), with six estimated parameters. The likelihood ratio test indicated that these two models are similar (X^2 = 9.83, df = 4, p = 0.05) and therefore indistinguishable.

Table 5: Models selection for apparent survival (φ) and recapture (ρ) of mark-recapture data from time groups released into three enclosures over 12 encounter occasions.

Model	AICc	ΔAICc	AICc Weights	Number Parameters
φ (g) ρ (g)	**479.61**	**0**	**0.546**	**10**
φ (.) ρ (g)	**480.30**	**0.69**	**0.386**	**6**
φ (g) ρ (.)	485.65	6.04	0.027	6
φ (t) ρ (g)	485.69	6.08	0.026	16
φ (.) ρ (.)	486.97	7.37	0.014	2
φ (t) ρ (.)	492.76	13.15	0.001	12
φ (g) ρ (t)	493.63	14.02	0	16
φ (.) ρ (t)	494.12	14.51	0	12
φ (t) ρ (t)	500.91	21.31	0	21
φ (.) ρ (g*t)	552.77	73.16	0	56
φ (g) ρ (g*t)	558.46	78.85	0	60
φ (t) ρ (g*t)	574.77	95.16	0	66
φ (g*t) ρ (g)	575.91	96.30	0	59
φ (g*t) ρ (.)	576.14	96.53	0	55
φ (g*t) ρ (t)	599.12	119.51	0	65
φ (g*t) ρ (g*t)	746.14	266.53	0	105

Models with a ΔAICc value < 2 were considered "best" fit models, indicated in bold
Model notation: φ survival, ρ recapture, (g) environmental group, (t) time spent trapping, (.) no effect.

Survival and recapture were affected by time spent in environments (SS, SL, CS, and CL) (Table 7). Probability estimates for apparent survival and recapture of

individuals from the SL environment were S = 0.95 (SE = 0.02) and R = 0.93 (SE = 0.02) and SS environment were S = 0.80 (SE = 0.07) and R = 0.79 (SE = 0.08), respectively. For individuals from a CL and CS environment, the apparent survival and recapture probability estimates were S = 0.95 (SE = 0.02) and R = 0.98 (SE = 0.01) and S = 0.44 (SE = 0.11) and R = 0.79 (SE = 0.05), respectively. Survival and recapture probability estimates for all time groups were not significantly different (Kruskal-Wallis df = 4, $p_{survival;\ recapture} = 0.41$). Probability estimates for survival were similar among individuals from SS, SL, and CL environments (Figure 3). The models suggested that individuals from CS and wild environments had similarly high survival estimates, while individuals from CL and wild environments showed similarly low recapture probability estimates.

Table 6: Mean estimates of apparent survival and recapture probabilities with associated standard error (SE) and 95% confidence intervals (CI) for time groups over 12 encounter occasions. Survival and recapture estimates produced from group only Cormack-Jolly-Seber model (φ(g) ρ(g)) in program MARK.

Time groups	N [a]	Estimate	SE	95% CI
Apparent Survival				
Simple long	12	0.95	0.02	0.91 - 0.97
Simple short	11	0.93	0.02	0.88 - 0.96
Complex long	8	0.95	0.02	0.89 - 0.98
Complex short	11	0.98	0.01	0.96 - 0.99
Wild	13	0.97	0.01	0.94 - 0.99
Apparent Recapture				
Simple long	12	0.80	0.07	0.64 - 0.90
Simple short	11	0.79	0.08	0.59 - 0.91
Complex long	8	0.44	0.11	0.25 - 0.66
Complex short	11	0.79	0.05	0.68 - 0.87
Wild	13	0.59	0.06	0.47 - 0.71

[a] Sample size (N) represents number of voles marked and initially released

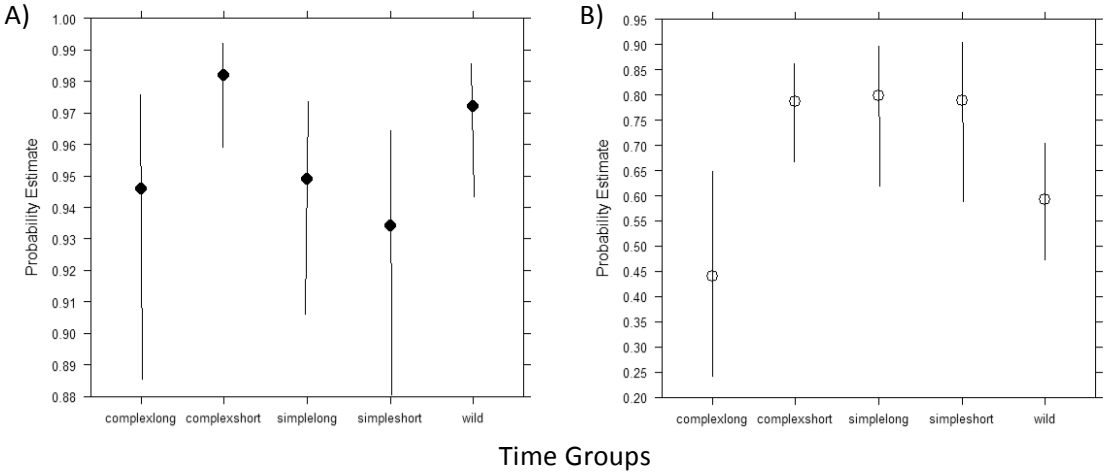

Figure 3: Mean estimates for apparent A) survival and B) recapture probabilities of time groups (SL, SS, CL, CS, and wild).

Discussion

My results suggest that survival of reintroduced voles was dependent on exposure to environmental complexity during captivity. Voles temporarily housed in a complex environment as adults showed higher survival rates than voles in a simple environment, but was not statistically significantly different. In fact, individuals from the complex environment had similar survival rates as individuals from the wild. This reinforces studies that suggest additional biologically relevant elements in captivity can improve reintroduction survival. My study highlights another important factor that may affect survival in the wild after being reintroduced: amount of time spent in a captive environment.

Environment in captivity.

Probability estimates of survival for environment groups (simple, complex, wild) supported my prediction that individuals from a complex captive environment would have similar survival rates as individuals from the wild. There was, however, no statistically significant difference between the environmental group estimates for survival. A larger sample size of individuals housed in a complex environment may have resulted in a difference between complex and simple groups.

Aaltonen et al. (2009) clearly showed decreased survival of captive-born marmots when compared to wild-born marmots and suggested this may result from lack of crucial behaviors after being in captivity. Therefore one hypothesis that may explain

survival differences is behavioral deficiencies resulting from captivity (Green et al., 2005, Mathews et al., 2005). The captive environment lacks many opportunities to express naturalistic behaviors, such as foraging, and socializing. Behaviors that do develop in response to the captive environment may not be advantageous in the wild. Inability to perform naturalistic behaviors such as foraging or habituation to stimuli in captivity may lead to abnormal behaviors (Beisner and Isbell, 2008) and subsequent low survival rates in the wild (Bremner-Harrison, 2004). My results showed that environmental complexity maintained somewhat higher survival rates. The complex environment provided voles an opportunity to perform foraging tasks and build elaborate nests and exposure to unpredictability through rotating novel objects. Use of complex elements is aimed at improving the quality of the captive environment to encourage performance of naturalistic activities. Allowing animals to exert some control over their environment (Buchanan-Smith, 1997, Carlstead, 1996), by providing the option to modify their environment (Newberry, 1995), increases an animal's ability to cope with challenges (Young, 2003) and promotes expression of species-specific behaviors (Bassett and Buchanan-Smith, 2007). It has also been shown that complexity in captivity may maintain activity levels and natural behavioral range (Burrell and Altman, 2006, Olsson and Dahlborn, 2002) that result in improved motor skills (Prior and Sachser, 1995).

Another hypothesis for survival differences across groups is stress from the captive environment, handling, or movement between different environments over a

short period of time. Some studies suggest that the rapid transport and handling of individuals create chronic and acute stress that can decrease survival in the wild (Dufour et al., 2011, Molony et al., 2006, Teixeira et al., 2007,). The captive environment has been suggested to be a source of stress on animals (Morgan and Tromborg, 2007), but complexity in captivity reduces stress (Newberry, 1995). Black-footed ferrets and European minks (*Mustela lutreola*) benefited more when housed in a semi-natural pre-release environment (Biggins et al., 1998, Maran et al., 2009), and this method is recommended to reduce stress from being released into a novel environment.

Physiological change can affect survival in the wild because it can alter behaviors (e.g. social or spatial) or ability to uptake nutrients. Unpublished data suggest that voles held in captivity temporarily and over generations have lower testosterone levels than wild individuals (Franklin, unpublished MS). Thus, survival differences between sexes in reintroduced voles should be examined. Any effects on body condition caused by the captive environment can minimize survival (Champagnon et al., 2012, Green et al., 2005). If this were the case, then the complex environment then maintains a healthy physiology through increased activities that promote survival in the wild.

In my study, I used adult voles taken from the wild and housed in captivity for more than one month. The use of adult versus juvenile animals in reintroduction studies has not before been examined, yet it is important to understand how captivity affects reintroduction of all age groups. Stoinski and Beck (2004) suggest that an adult exposed

to complexity after developing in a non-complex environment does not gain behavioral benefits but rather exposure to complexity at a young age may positively affect their survival in the wild. My study showed that adult voles are still affected by temporary housing in captivity, and the type of environment they are exposed to can affect their survival differently. This would reinforce the use of complex environments at all age groups when animals are being held in captivity.

Both environment and time groups also affected recapture probability estimates. Voles from both captive environments (simple and complex) had higher recapture rates than their wild counterparts. Effects on recapture rates would suggest differences in trapping ability of released animals that could bias survival estimates. Wild (control) voles were then considered to be trap-shy even though mark-recapture analysis showed that they had the highest survival probabilities. No difference in recapture probability in individuals from the captive environments indicates their habituation and fearlessness to the trap. However, differences in survival rates between captive environments would suggest that there was no bias in estimating survival because individuals had the same likelihood of being caught.

Time spent in captive environment.

Teixeira et al. (2007) asked whether there is an optimal length of time in captivity that may maintain high survival when released. My results showed that voles housed in a complex environment for less than 1.5 months may promote survival

probability similar to wild (control) voles after release into the wild. Estimates were not significantly different for survival between time (SS, SL, CS, CL) and wild groups, but a larger sample size may strengthen observable trends. My prediction that individuals from a CL environment would have similar survival probabilities as individuals from the CS environment was not supported; instead, they had lower survival probability estimates similar to the simple environment. This difference in survival may be attributed to the smaller sample size in the CL group (n = 8). In any case, this suggests that being housed in a complex environment for less than 1.5 months might maintain important traits and reduce stress, resulting in survival rates similar to those of released wild (control) counterparts. Molony et al. (2006) experimentally showed that relocated hedgehogs (*Erinaceus europaeus*) were more likely to survive longer after release if they were housed in captivity for a minimum of one month; shorter periods would decrease survivorship. Their explanation for this was that captivity for less than one month would cause stress to the individuals from rapid changes in environment and handling by humans, or would not provide sufficient time for the individuals to gain weight before release. My results showed that individuals from a SS environment had the lowest survival probability and may suggest that the simple environment decreases survival if housed for less than 1.5 months, possibly because of stress.

In my study, individuals from a CL environment showed similar survival probabilities as those from a SL and SS environment. This may have resulted from

behavioral deficiencies that arose after being housed in captivity (whether complex or simple) for greater than 1.5 months. Length of time in any type of captive environment may equally become predictable or greatly affect physiology thereby reducing survival probability (Bassett and Buchnan-Smith, 2007). Relocated elephants had initially different foraging time budgets than wild elephants (*Loxodonta africana*) after release, but differences disappeared over time (Pinter-Wollman et al., 2009). This may support the idea that minimal time in captivity maintains high survival rates because behavioral deficiencies can be reversed more quickly.

The difference in survival between individuals from a SS and CS environment may be because the complexity in the environment minimized any stressors of movement from the wild to captivity to the wild again. Molony et al. (2006) suggested that animals that were relocated within six days in captivity had the lowest survival rates when released into the wild, possibly as a result of stress. By contrast, my study suggests that wild animals (my reintroduction control) had the highest survival rates, and they were maintained in captivity for less than 10 days, although they were minimally handled. Stress has been linked to lower survival probability after reintroduction, but certain steps can be taken to reduce stressors. Use of pre-release exposure (Letty et al., 2000) and soft release (Bright and Morris, 1994) methods can minimize stress during transfer, and this study used those procedures. Although I did not test for stress hormone differences, the use of a soft release method and high survival in wild animals,

suggest that survival differences between groups in this study were not a result of stress.

Comparison of SS with CS and CL with SL individuals showed that environment has a strong effect on survival probability if housed for less than 1.5 months, but after 1.5 months effects of environments become indistinguishable. This may be due to the season in which individuals were brought into captivity and assigned to a time group. Individuals caught at the beginning of summer just after snowmelt may not have been in the same body condition as individuals caught during mid-summer, compromising survival in the wild. If this were the case, survival results would suggest instead that the captive environment does not affect an individual's survival probability if captured early in the summer, but the captive environment strongly affects survival of individuals captured in late summer after natural emergence from winter. The difference in effects of environment may be explained by the inability of the complex environment to provide resources or experiences that could have enhanced an individual's physiology after emergence from winter.

Conclusions

Examining survivorship of individuals housed in captivity under varying levels of complexity is only the first step in understanding important mechanisms involved in affecting survival in the wild. The IUCN guidelines for reintroduction state that captive

individuals should be maintained in housing conditions that enable learning and experiences that maintain survival probabilities approximately the same as wild counterparts (IUCN, 1995). This study provides support for individuals undergoing translocation to be housed in a captive environment that provides complex elements, survival in the wild. I also highlight that time in captivity should be for a short period of time (dependent on species) to increase survival probabilities after release.

Chapter IV

Conservation Implications: How Do Differences in Foraging Behavior Affect Survival Probability in the Wild?

Relocating species for conservation management is successful when behavioral theory has been applied to understand species' needs to fulfill physiological and psychological requirements (Champagon et al., 2012, Stoinski and Beck, 2004). In this unique study, I have combined behavioral investigation with applied conservation research. I examined how changes in foraging behavior in response to environmental complexity in captivity relate to survival probability in the wild.

I found that the elements of environmental complexity used in this study were not sufficient to significantly change foraging behaviors in individuals housed in a simple or complex environment. However, examining individuals within each environment presented significant differences in sex-specific foraging behavior and response to unpredictability. Olsson and Dahlborn (2002) reviewed studies conducted on effects of cage structure and complexity on behavioral responses to maze and open field tests in mice. Mice were more active and exploratory during these tests, indicating a healthy well-being and increased ability to cope with stress from novelty. While these mice

showed higher activity and possible boldness behaviors (from decreased fearfulness) we do not fully understand how those behavioral repertoires affect survival in the wild.

Mark-recapture analysis of survival after being reintroduced to the wild showed that voles temporarily housed in a complex environment had a slightly higher survival probability than voles housed in a simple environment. Voles housed in a complex environment for less than 1.5 months had the highest survival rate across all groups.

Based on behavioral results, one cause for these survival differences is maintenance of behavioral response to unpredictability in food availability and motivation for exploration. Time spent in the captive environment also influenced survival in the wild, and stress may be one hypothesis that explains this difference (but not tested in this study). Transport to a novel environment temporarily can create a stressful situation for individuals that can decrease appropriate response to the wild environment (Dufour et al., 2011, Molony et al., 2006). The captive environment can, over time, deteriorate or select for behavioral traits that are adaptive to the captive environment but maladaptive in the wild (Stoinski and Beck, 2004). Therefore, time can be important during translocation of individuals. However, based on behavioral results, effect of time in captivity can differ as a result of amount of environmental complexity.

My study shows that even environmental complexity can become predictable (given time) and promote behaviors that affect survival in the wild. Simple housing for greater than 1.5 months can be more effective at minimizing stress when complexity

cannot be provided because factors affecting stress are reduced by environmental predictability (Bassett and Buchanan-Smith, 2007) and individuals may be better prepared to react appropriately to a changing environment. Behavioral data suggest that individuals housed for greater than 1.5 months minimize movements in a novel environment, and I expect that this would decrease survival probability in the wild because reduced locomotion and inability to locate food is fatal. However, frequency and duration of time spent eating showed no significant difference. This would suggest that individuals do not necessarily locate or were motivated to locate food resources, and this was represented in the lower survival rates of individuals housed in an environment for greater than 1.5 months.

This study supports the finding that environmental complexity within captivity does have its limitations and that not all foraging behaviors are altered in adult voles. However, not all behaviors in a repertoire need to be changed to affect survival differently.

The results of this study can aid conservation biologists and managers in their decisions to create naturalistic captive environments that will enhance reintroduction efforts and population growth and protect species diversity.

References

Aaltonen, K., Bryant, A.A., Hostetler, J.A., and Madan, K.O. (2009). Reintroducing Endangered Vancouver Island Marmots: Survival and Cause-Specific Mortality Rates of Captive-Born Versus Wild-Born Individuals. *Biological Conservation 142*:2181-2190

Akaike, H. (1973). *Information Theory as an Extension of the Maximum Likelihood Principle*. Inc Petrov B.N., Csaki, F., editors. Second International Symposium on Information theory. Akademiai Kiado. New York: Academic Press. 267-281

Altmann, J. (1974). Observational Study of Behavior: Sampling Methods. *Behaviour 48*:227-260.

Angeloni, L., Schlaepfer, M.A, Lawler, J.J., and Crooks, K.R. (2008). A Reassessment of the Interface between Conservation and Behavior. *Animal Behavior 75*:731-737

Anthony, L.L and Blumstein, D.T. (2000). Integrating Behavior into Wildlife Conservation: The Multiple Ways that Behavior Can Reduce N_e. *Biological Conservation 95*:303-315

Archer, J. E. and Blackman, D. E. (1971). Prenatal Psychological Stress and Offspring Behavior in Rats and Mice. *Developmental Psychobiology 4*: 193–248

Arcis, V. and Desor, D. (2003). Influence of Environment Structure and Food Availability on the Foraging Behaviour of the Laboratory Rat. *Behavioral Processes 60*:191-198

Bassett, L. and Buchanan-Smith, H.M. (2007). Effects of Predictability on the Welfare of Captive Animals. *Applied Animal Behaviour Science 102*:223-245

Baumans, V. (2011). Environment Enrichment for Laboratory Rodents and Rabbits: Requirements of Rodents, Rabbits, and Research. *ILAR Journal 46*:162-170

Beck, B. B. and Castro, M. I. (1994). Environments for endangered primates. In E. F. Gibbons, E. J. Wyers, E. Waters,&E.W. Menzel (Eds.), *Naturalistic environments in captivity for animal behavior research* 259–270. Albany: State University of New York Press

Beck, B. B., Castro, M. I., Stoinski, T. S., and Ballou, J. D. (2002). The Effects of Prerelease Environments and Postrelease Management on Survivorship in Reintroduced

Golden Lion Tamarins. In D. G. Kleiman & A. B. Rylands (Eds.), *Lion tamarins: Biology and Conservation* 283–300. Washington, DC: Smithsonian Institute

Beisner, B.A. and Isbell, Lynne. A. (2008). Ground Substrate Affects Activity Budgets and Hair Loss in Outdoor Captive Groups of Rhesus Macaques (*Macaca mulatta*). *American Journal of Primatology 70*:1160-1168

Beissinger, S.R., Clemmons, J.R., and Buchholz, R. (1997). Integrating Behavior into Conservation Biology: Potentials and Limitations. *Behavioral Approaches to Conservation in the Wild.* Cambridge University Press, Cambridge 23-47

Berger-Tal, O., Polak, T., Oron, A., Lubin, Y., Kotler, B.P., and Saltz, D. (2011). Integrating Animal Behavior and Conservation Biology: A Conceptual Framework. *Behavioral Ecology 22*:236-239

Biggins, D. E., Godbey, J. L., Hanebury, L. R., Luce, B., Matchett, M. R. , and Vargas, A. (1998). The Effect of Rearing Methods on Survival of Reintroduced Black-Footed Ferrets. *Journal of Wildlife Management* 62:643–653

Blumstein, D.T. and Daniel, J.C. (2007). Quantifying *Behavior the JWatcher Way*. Sunderland, MA: Sinauer Associates.

Boonstra, R., Xia, X., and Pavone, L. (1993). Mating System of the Meadow Vole, *Microtus pennsylvanicus*. *Behavioral Ecology 4*(1):83-89

Bowers, M.A., Gregario, K., Brame, C.J., Matter, S.F., and Dooley Jr, J.L. (1996). Use of Space and Habitats by Meadow Voles at the Home Range, Patch and Landscape Scales. *Oecologia 105*:107-115

Bremner-Harrison, S., Prodohl, P.A., Elwood, R.W. (2004). Behavioural Trait Assessment as a Release Criterion: Boldness Predicts Early Death in a Reintroduction Programme of Captive-Bred Swift Fox (Vulpes velox). *Animal Conservation 7*:313–320.

Bright, P.W. and Morris, P.A. (1994). Animal Translocation for Conservation Performance of Dormice in Relation to Release Methods, Origin and Season. *Journal of Applied Ecology 31*:699-708

Britt, A. (1998). Encouraging Natural Feeding Behavior in Captive-Bred Black and White Ruffed Lemurs (*Varecia variegata variegata*). *Zoo Biology 17*:379–392

Buchholz, R. (2007). Behavioral Biology:An Effective and Relevant Conservation Tool. *TRENDS in Ecology and Evolution 22*(8):401-407

Burrell, A.M. and Altman, J.D. (2006). The Effect of the Captive Environment on Activity of Captive Cotton-Top Tamarins (*Saguinus oedipus*). *Journal of Applied Animal Welfare Science 9*(4):269-276

Burnham, K.P., and Anderson, D.R. (2002). *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.

Carlstead, K. (1996). Effects of Captivity on the Behaviour of Wild Mammals. In: Kleiman DG, Allen ME, Thompson KV, Lumpkin S, editors. *Wild Mammals in Captivity: Principles and Techniques*. London: University of Chicago Press 317-333

Carlstead, K. and Shepherdson, D. (1994). Effects of Environmental Enrichment on Reproduction. *Zoo Biology 13*:447-458

Caro, T. (1999). The Behaviour-Conservation Interface. *TREE 14*(9):366-369

Caro, T. (2007). Behavior and Conservation: A Bridge Too Far? *TRENDS in Ecology and Evolution 22*(8):396-400

Champagon, J., Guillemain, M., Elmberg, J.,Massez, G., Cavallo, F., and Gauthier-Clerc, M. (2012). Low Survival After Release into the Wild: Assessing "The Burden of Captivity" on Mallard Physiology and Behavior. *European Journal of Wildlife Research 58*(1):255-267

Clulow, F.V. and Mallory, F.F. (1970). Oestrus and Induced Ovulation in the Meadow Vole, *Microtus pennsylvanicus*. *Journal of the Society for Reproductive Fertility 23*:341-343

Cooch, E. and White, G. C. (2006). *Program MARK: a gentle introduction*. 5th Edition. Cornell University, Ithaca.

Cooper, J.J., Odberg, F., and Nicol, C.J. (1996). Limitations on the Effectiveness of Environmental Improvement in Reducing Stereotypic Behaviour in Bank Voles (*Clethrionomys glareolus*). *Applied Animal Behaviour Science 48*(3-4):237-248

Cormack, R.M. (1964). Estimates of Survival from the Sighting of Marked Animals. *Biometrika 51*:429-438

Cunningham, A. A. (1996). Disease Risks of Wildlife Translocations. *Conservation Biology 10*: 349–353

Daan, S. and Tinbergen, J. M. (1997). Adaptation of life histories. In *Behavioural Ecology: an Evolutionary Approach* (eds. J. R. Krebs and N. B. Davies), pp. 311–333. Blackwell, Oxford.

Darwin, C. (1868). *The Variation of Animals and Plants Under Domestication*. London: John Murray. 1st ed., 2nd issue.

Dufour, V., Sueur, C., Whiten, A., and Buchanan-Smith, H.M. (2011). The Impact of Moving to a Novel Environment on Social Networks, Activity and Wellbeing in Two New Primates. *American Journal of Primatology 73*:1-10

Endler, J.A. (1986). *Natural Selection in the Wild*. Monographs in Population Biology 21

Fischer, J. and Lindenmayer, D.B. (2000). An Assessment of the Published Results of Animal Relocations. *Biological Conservation 96*(1):1-11

Franklin, K. (2011). The Effects of the Captive Environment on Total Testosterone Levels in Wild Caught Meadow Voles. Unpublished

Fortier, G.M, and Tamarin, R.H. (1998). Movement of Meadow Voles in Response to Food and Density Manipulations: A Test of the Food-Defense and Pup-Defense Hypotheses. *Journal of Mammalogy 79*(1):337-345

Ginnett, T.F. and Demment, M.W. (1997). Sex Differences in Giraffe Foraging Behavior at Two Spatial Scales. *Oecologia 110*(2):291-300

Gittleman, J.L. and Thompson, S.D. (1988). Energy Allocation in Mammalian Reproduction. *American Zoologist 28*:863-875

Glatston, A.R., Geilvoet-Soeteman, E., Hora-Pecek, E., Van Hooff, J.A.R.A.M. (1984). The Influence of the Zoo Environment on Social Behavior of Groups of Cotton-Topped Tamarins, *Saguinus oedipus Oedipus*. *Zoo Biology 3*(3):241-253

Green, A.J., Fuentes, C., Figuerola, J., Viedma, C., Ramon, N. (2005). Survival of Marbled Teal (*Marmaronetta angustirostris*) Released Back into the Wild. *Biological Conservation 121*:595–601

Griffin, A.S., Blumstein, D.T., and Evans, C.S. (2000). Training Captive-Bred or Translocated Animals to Avoid Predators. *Conservation Biology 14*:1317-1326

Griffith, B., Scott, J.M., Carpenter, J.W., Reed, C. (1989). Translocation as Species Conservation Tool: Status and Strategy. *Science 245*:477–480

Hannah, A. C. and Brotman, B. (1990). Procedures for Improving Maternal Behavior in Captive Chimpanzees. *Zoo Biology* 9:233–240

Hansen, L.T., and Berthelsen, H. (2000). The Effect of Environmental Enrichment on the Behavior of Caged Rabbits (*Oryctolagus cuniculus*). *Applied Animal Behaviour 68*:163-178

Hosey, G.R. (2005). How Does the Zoo Environment Affect the Behaviour of Captive Primates? Applied *Animal Behaviour Science 90*:107-129

Howe, H.F., Zorn-Arnold, B., Sullivan, A., and Brown, J.S. (2006). Massive and Distinctive Effects of Meadow Voles on Grassland Vegetation. *Ecology 87*(12):3007-3013

IUCN (1995). Guidelines for Re-introductions. Re-introduction Specialist Group, Species Survival Commission. Gland, Switzerland:IUCN

Jensen, P. and Toates, F.M. (1993). Who Needs 'Behavioural Needs'? Motivational Aspects of the Needs of Animals. *Applied Animal Behaviour Science 37*:161-181

Jolly, G.M. (1965). Explicit Estimates from Capture-Recapture Data with Both Death and Immigration-Stochastic Model. *Biometrika 52*:225-247

Jones, E.N. (1990). Effects of Forage Availability on Home Range and Population Density of *Microtus pennsylvanicus. Journal of Mammalogy 71*(3):382-289

Jule,K.R., Leaver, L.A., and Lea, S.E.G. (2008). The Effects of Captive Experience on Reintroduction Survival in Carnivores: A Review and Analysis. *Biological Conservation 141*(2):355-363

Keller, B.L., and Krebs, C.J. (1970). Microtus Population Biology; III. Reproductive Changes in Fluctuating Populations of *M.ochrogaster* and *M.pennsylvanicus* in Southern Indiana. *Ecological Monographs 40*(3):263-294

Kelley, J.L., Magurran, A.E., Macias-Garcia, C. (2005). The Influence of Rearing Experience on the Behavior of an Endangered Mexican Fish *Skiffia multipunctata*. *Biological Conservation 122*:223-230

Key, C. and Ross, C. (1999). Sex Differences in Energy Expenditure in Non-human Primates. *Proceedings of the Royal Society of London 266*:2479-2485

Kitchen, A.M. and Martin, A.A. (1996). The Effects of Cage Size and Complexity on the Behaviour of Captive Common Marmosets, *Callithrix jacchus* jacchus*. Laboratory Animals 30*:317-326

Kleiman, D.G. (1989). Reintroduction of Captive Mammals for Conservation. *BioScience 39*(3):152-161

Kleiman, D.G., Stanley Price, M.R., and Beck, B.B. (1994). Criteria for Reintroductions. In:Olney, P.J.S., Mace, G.M., Feistner, A.T.C. (Eds.), *Creative Conservation: Interactive Management of Wild and Captive Animals*. Chapman and Hall, London 287-303

Kohane, M.J., and Parsons, P.A. (1988). Domestication: Evolutionary Change Under Stress. *Evolutionary Biology 23*:31-48

Krebs, C.J., Keller, B.L., and Tamarin, R.H. (1969). *Microtus* Population Biology: Demographic Changes in Fluctuating Populations of *M.ochrogaster* and *M.pennsylvanicus* in Southern Indiana. *Ecology 50*:587-607

Lebreton, J. D., Burnham, K. P., Clobert, J., and Anderson, D. R. (1992). Modeling survival and Testing Biological Hypotheses Using Marked Animals: A Unified Approach with Case Studies. *Ecological Monographs 62*:67–118

Letty, J., Marchandeau, S., Clobert, J., and Aubineau, J. (2000). Improving Translocation Success: an Experimental Study of Anti-stress Treatment and Release Method for Wild Rabbits. *Animal Conservation 3*:211-219

Lindroth, R.L., and Batzli, G.O. (1984). Food Habits of Meadow Vole (*Microtus pennsylvanicus*) in Bluegrass and Prairie Habitats. *Journal of Mammalogy 65*(4):600-606

Low, B.S. (2000). *Why Sex Matters: A Darwinian Look at Human Behavior*. Princeton University Press, Princeton, NJ

Madison, D.M. (1980). Space Use and Social Structure in Meadow Voles, *Microtus pennsylvanicus*. *Behavioral Ecology and Sociobiology 7*:65-71

Maran, T., Podra, M., Polma, M., Mcdonald, D., (2009). The Survival of Captive-Born Animals in Restoration Programmes – Case Study of the Endangered European Mink *Mustela lutreola*. *Biological Conservation 142:*1685–1692.

Mathews, F. Orros, M., McLaren, G., Gelling, M., and Foster, R. (2005). Keeping Fit on the Ark: Assessing the Suitability of Captive-Bred Animals. *Biological Conservation 121*(4):569-577

McPhee, M.E. (2002). Intact Carcasses as Enrichment for Large Felids: Effects on On- and Off-Exhibit Behaviors. *Zoo Biology 21*:37-47

McPhee, M.E. (2004a). Generations in Captivity Increases Behavioral Variance: Considerations for Captive Breeding and Reintroduction Programs. *Biological Conservation 115*:71-77

McPhee, M.E. (2004b). Morphological Change in Wild and Captive Oldfield Mice *Peromyscus polionotus subgriseus*. *Journal of Mammalogy 85*(6):1130-1137

McPhee, M.E., and Silverman, E.D. (2004). Increased Behavioral Variation and the Calculation of Rlease Numbers for Reintroduction Programs*. Conservation Biology 18(*3):705-715

McPhee M.E., and Carlstead K. (2010). The Importance of Maintaining Natural Behaviors in Captive Mammals. In: Kleiman DG, Thompson KV, Baer CK, editors. *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Chicago, IL: University of Chicago Press: 303–313

Meehan, C.L. and Mench, J.A. (2007). The Challenge of Challenge: Can Problem Solving Opportunities Enhance Animal Welfare? *Applied Animal Behaviour Science 102*: 246-261

Miller, B., Biggins, D., Wemmer, C., Powell, R., Calvo, L., Hanebury, L., and Wharton, T. (1990). Development of Survival Skills in Captive- Raised Siberian Polecats (*Mustela eversmanni*) II:Predator Avoidance. *Journal of Ethology 8*:95-104

Molony, S.E., Dowding, C.V., Baker, P.J., Cuthill, I.C., and Harris, S. (2006). The Effect of Translocation and Temporary Captivity on Wildlife Rehabilitation Success: An Experimental Study Using European Hedgehogs (*Erinaceus europaeus*). *Biological Conservation 130*(4):530-537

Moore, C. L. (1984). Maternal Contributions to the Development of Masculine Sexual Behavior in Laboratory Rats. *Developmental Psychobiology 17*:347–356

Morgan, K.N. and Tromborg, C.T. (2007). Sources of Stress in Captivity. Applied Animal Behaviour Science 102:262-302

Myers, J.H. and Krebs, C.J. (1971). Genetic, Behavioral, and Reproductive Attributes of Dispersing Field Voles *Microtus pennsylvanicus* and *Microtus ochrogaster*. *Ecological Monographs 41*(1):53-78

National Research Council. (1996). "2 Animal Environment, Housing, and Management." *Guide for the Care and Use of Laboratory Animals*. Washington, DC: The National Academies Press. 1. Print.

Newberry, RC. (1995). Environmental Enrichment: Increasing the Biological Relevance of Captive Environment. *Applied Animal Behaviour Science 44*:229-243.

Novak, M.A., O'Neill, P., Beckley, S.A., Suomi, S.J. (1994). Naturalistic Environments for Captive Primates. In: Gibbons EF editor. *Naturalistic environments in captivity for animal behaviour research*. Albany: State University of New York Press. p 235-257

Odberg, F.O. (1987). The Influence of Cage Size and Environmental Enrichment on the Development of Stereotypies in Bank Voles (*Clethrionomys glareolus*). *Behavioral Processes 14*(2):155-173

Olsson, I.A.S. and Dahlborn, K. (2002). Improving Housing Conditions for Laboratory Mice: A Review of 'Environmental Enrichment'. *Laboratory Animals 36*:243-270

Pearce-Kelly, P., Mace, G.M., and Clarke, D. (1995) The Release of Captive Bred Snails (*Partula taeniata*) into a Semi-natural Environment. *Biodiversity and Conservation 4*(6): 645-663

Phillips, M.K., and Parker, W.T. (1988). Red Wolf Recovery: A Progress Report. *Conservation Biology 2*(2):139-144

Pinter-Wollman, N., Isbell, L.A., and Hart, L.A. (2009). Assessing Translocation Outcome: Comparing Behavioral and Physiological Aspects of Translocated and Resident African Elephants (*Loxodonta Africana*). *Biological Conservation 142*(5):1116-1124

Poole, T.B. (1998). Meeting a Mammal's Psychological Needs: Basic principles. In: Shepherdson DJ, Mellen JD, Hutchins M, editors. *Second nature: Environmental Enrichment for Captive Animals*. Washington: Smithsonian Institution. p 83-96

Prescott, M.J. and Buchanan-Smith, H.M. (2004). Cage Size for Tamarins in the Laboratory. *Animal Welfare 18*:747-752

Prior, H. and Sachser, N. (1995). Effects of Enriched Housing Environment on the Behaviour of Young Male and Female Mice in Four Exploratory Tasks. *Journal of Experimental Animal Science 37*:57–68

Pusenius, J. and Ostfeld, R.S. (2002). Mammalian Predator Scent, Vegetation Cover, and Tree Seedling Predation by Meadow Voles. *Ecography 25*(4):481-487

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Retrieved July 2011, from http://www.R-project.org

Rabin, L.A. (2003). Maintaining Behavioural Diversity in Captivity for Conservation: Natural Behaviour Management. *Animal Welfare 12*:85-94

Runyon, R.P. and Haber, A. (1980). *Fundamentals of Behavioral Statistics*. 4th Ed. Reading, MA: Addison-Wesley Publishing Company

Sanz, V. and Grajal, A. (1998). Successful Reintroduction of Captive-Raised Yellow-Shouldered Amazon Parrots on Margarita Island, Venezuela*. Conservation Biology 12*:430–441

Seber, G.A.F. (1965). A Note on the Multiple Recapture Census. *Biometrika 52*:249-259

Seddon, P.J. (1999). Persistence without Intervention: Assessing Success in Wildlife Reintroductions. *TREE 14*(12):503

Seddon, P.J., Armstrong, D.P., and Maloney, R.F. (2007). Developing the Science of Reintroduction Biology. *Conservation Biology 21*(2):303-312

Shier, D.M. and Owings, D.H. (2006). Effects of Predator Training on Behavior and Postrelease Survival of Captive Prairie Dogs (*Cynomys ludovicianus*). *Biological Conservation 132:*126–135

Simonoff JS. (2003). *Analyzing categorical data.* New York: Springer

Snyder, N. F. R. and Snyder, H.A. (1989). Biology and Conservation of the California Condor. *Current Ornithology 6*:175-263

Snyder, N.F.R., Derrickson, S.R., Beissinger, S.R., Wiley, J.W., Smith, T.B., Toone, W.D., and Miller, B. (1996). Limitations of Captive Breeding in Endangered Species Recovery. *Conservation Biology 10*:338-348

Soule, M.E. (1985). What is Conservation Biology? *BioScience 35*:727-734

Stephens, D.W. and Krebs, J.R. (1986). *Foraging theory*. Princeton, NJ: Princeton University Press

Stoinski, T.S. and Beck, B.B. (2004). Changes in Locomotor and Foraging Skills in Captive-Born, Reintroduced Golden Lion Tamarins (*Leotopithecus rosalia rosalia*). *American Journal of Primatology 62*(1):1-13

Stoinski, T.S., Beck, B.B., Bloomsmith, M.A., and Maple, T.L. (2003). A Behavioral Comparison of Captive-Born, Reintroduced Golden Lion Tamarins and Their Wild-Born Offspring. *Behavior 140*:137-160

Sundstrom, L.F. and Johnsson, J.I. (2001). Experience and Social Environment Influence the Ability of Young Brown Trout to Forage on Live Novel Prey. *Animal Behavior 61*:249-255

Sutherland, W.J. (1998). The Importance of Behavioural Studies in Conservation Biology. *Animal Behaviour 56*:801-809

Teixeira, C.P., Schetini De Azevedo, C., Mendl, M., Cipreste, C.F., and Young, R.J. (2007). Revisiting Translocation and Reintroduction Programmes:The Importance of Considering Stress. *Animal Behaviour 73*:1-13

Ulyan, M.J., Burrows, A.E., Buzzell, C.A., Raghanti, M.A, Marcinkiewicz, J.L., and Phillips, K.A. (2006). The Effects of Predictable and Unpredictable Feeding Schedules on the Behavior and Physiology of Captive Brown Capuchins (*Cebus paella*). *Applied Animal Behaviour Science 101*:154-160

Vargas, A. and Anderson, S.H. (1999). Effects of Experience and Cage Enrichment on Predatory Skills of Black-footed Ferrets (*Mustela nigripes). Journal of Mammalogy 80*(1):263-269

Veasey, J.S., Waran, N.K., and Young, R.J. (1996). On Comparing the Behaviour of Zoo Housed Animals with Wild Conspecifics as a Welfare Indicator. *Animal Welfare 5*(1):13-24

Wallace, M.P., Gosling, L.M., and Sutherland, W.J. (2000). Retaining Natural Behaviour in Captivity for Reintroduction Programmes. *Behaviour and Conservation*, Cambridge University Press, Cambridge 300-314

Watters, J.V. and Meehan, C.L. (2007). Different Strokes: Can Managing Behavioral Types Increase Post-Release Success? *Applied Animal Behaviour Science 102*:364-379

White, G.C. Program MARK, Colorado State University, Fort Collins, CO. Retrieved September 2011, from http://warnercnr.colostate.edu/~gwhite/mark/mark.htm

White Jr, T.H., Collazo, J.A., and Vilella, F.J. (2005). Survival of Captive-Reared Puerto Rican Parrots Released in the Caribbean National Forest. *The Condor 107*:424-432

Wolff, J.O. (2003). Laboratory Studies with Rodents: Facts or Artifacts? *American Institute of Biological Sciences 53*(4):421-427

Yamada, J. K. and Durrant, B. S. (1989). Reproductive Parameters of Clouded Leopards (*Neofelis nebulosa*). *Zoo Biology, 8*:223–231

Young, R.J. (2003). *Environmental Enrichment for Captive Animals*. UFAW Animal Welfare Series. London: Blackwell Science Ltd.